

18. Studies on Pearl-Oysters and Pearls.—I. The Structure of the Shell and Pearls of the Ceylon Pearl-Oyster (*Margaritifera vulgaris* Schumacher): with an Examination of the Cestode Theory of Pearl-Production. By H. LYSTER JAMESON, M.A., D.Sc., Ph.D., F.Z.S.

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(Plates XXXIII.–XLVII. and Text-figures 33–41.)

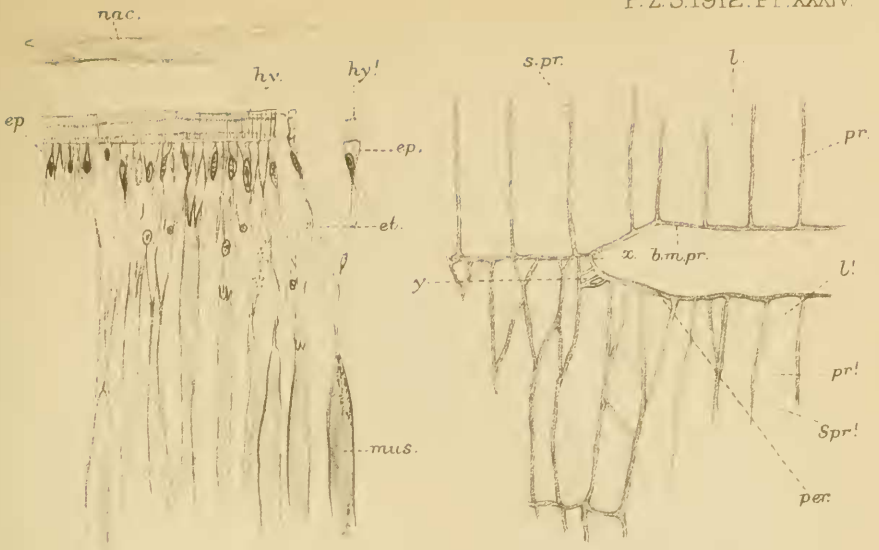
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(1) INTRODUCTION.

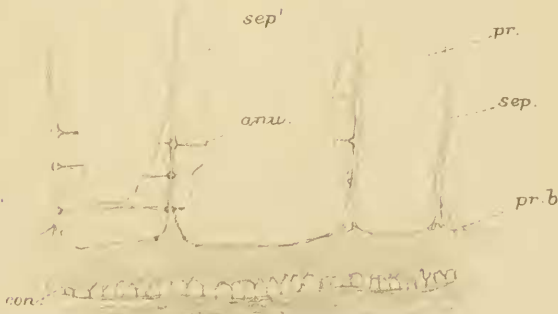
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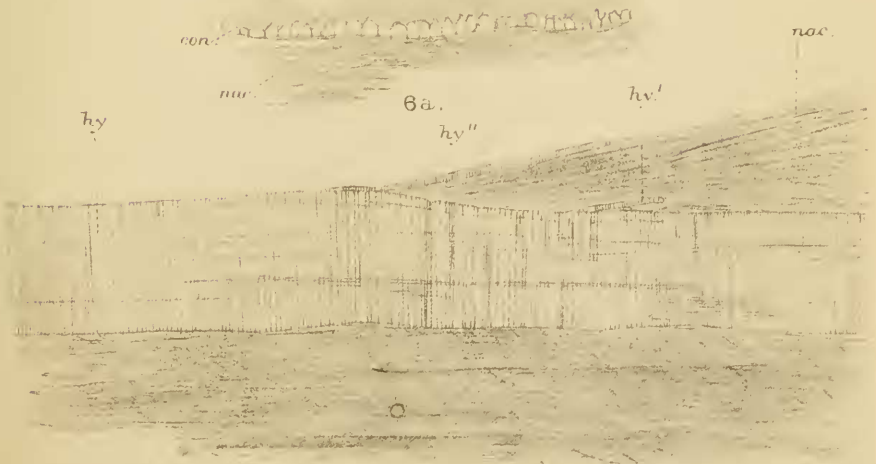
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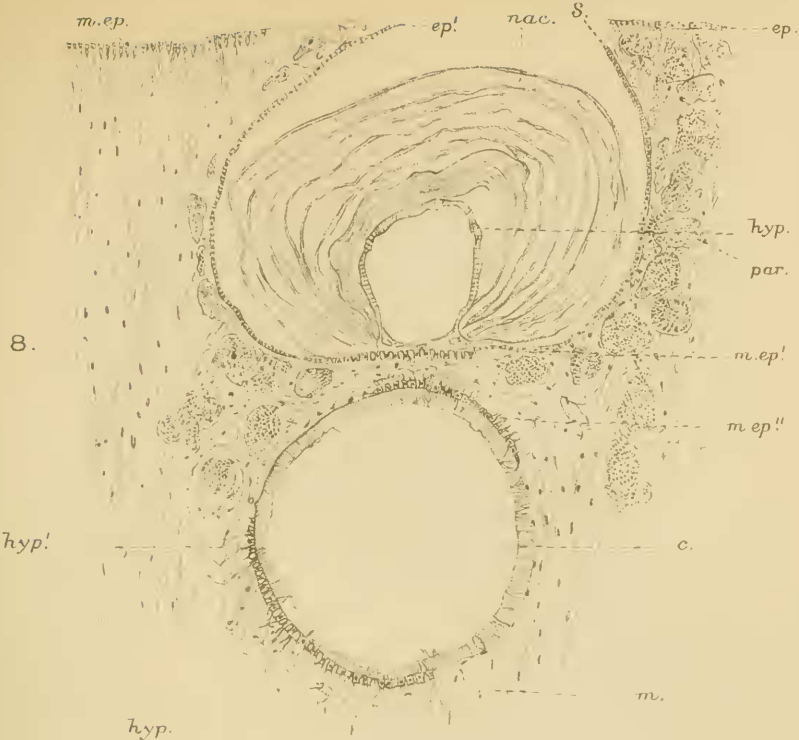
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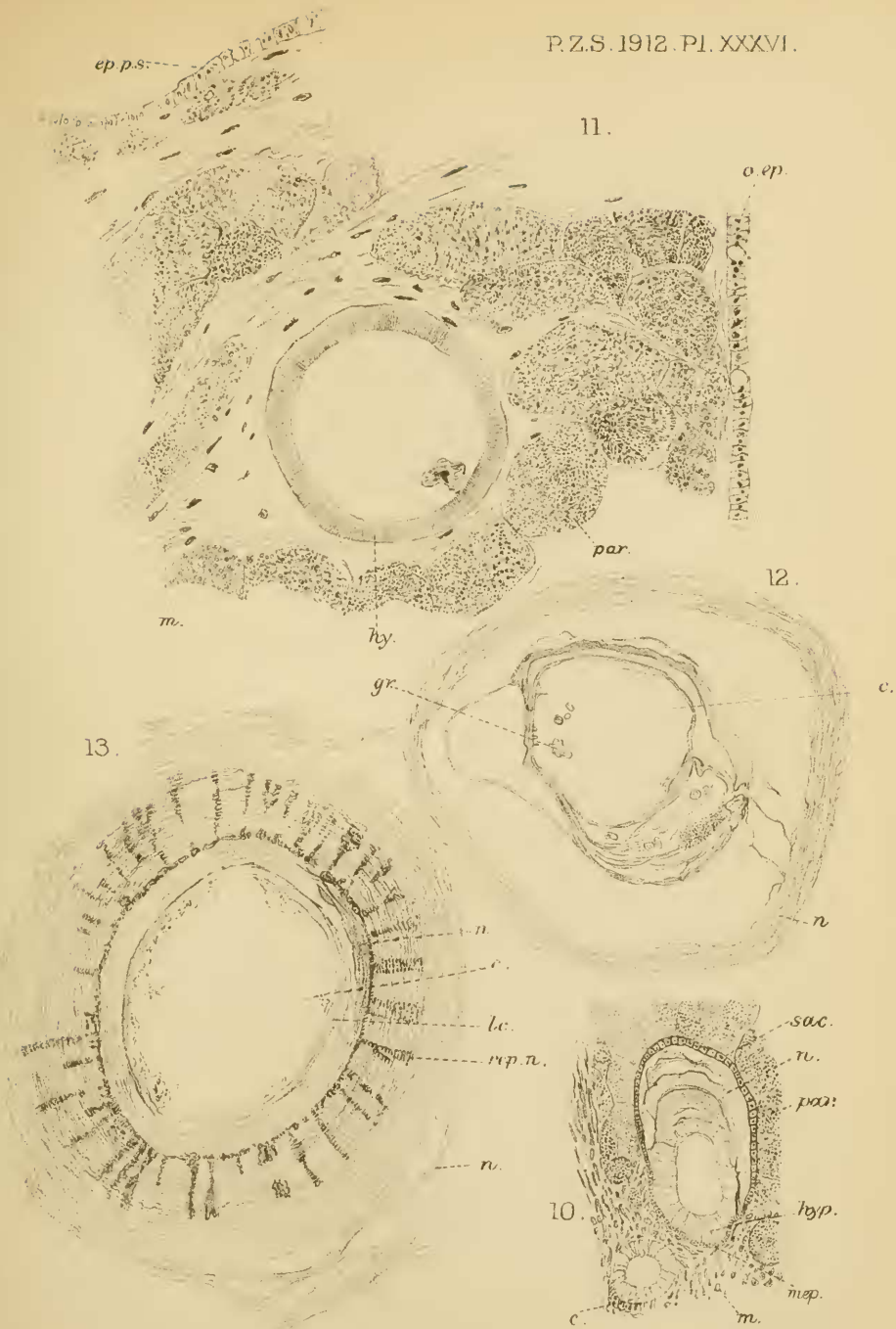
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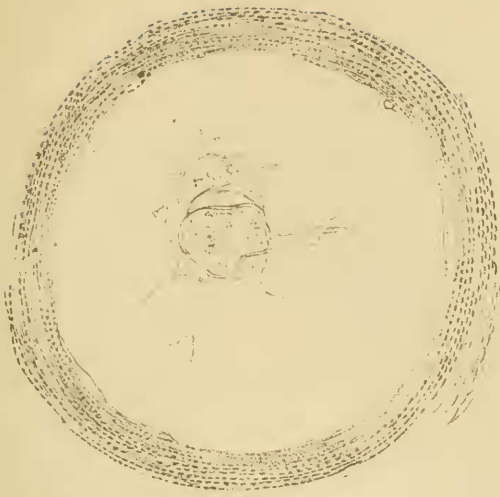
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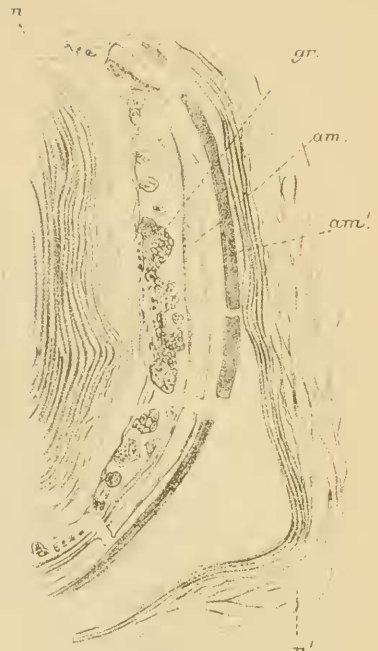
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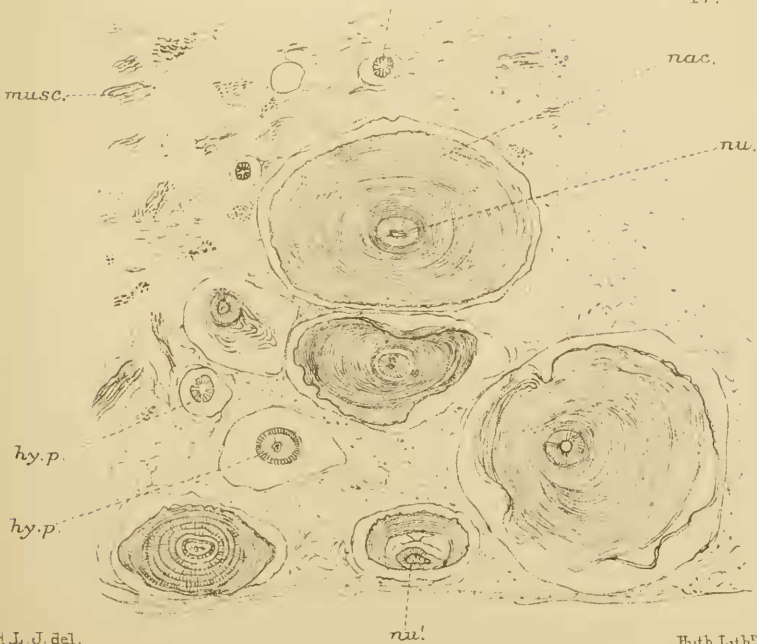


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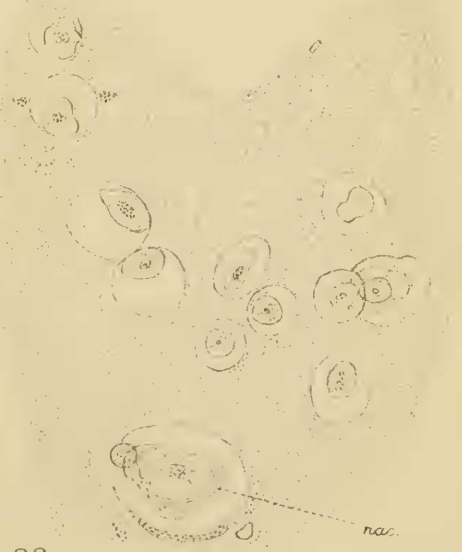
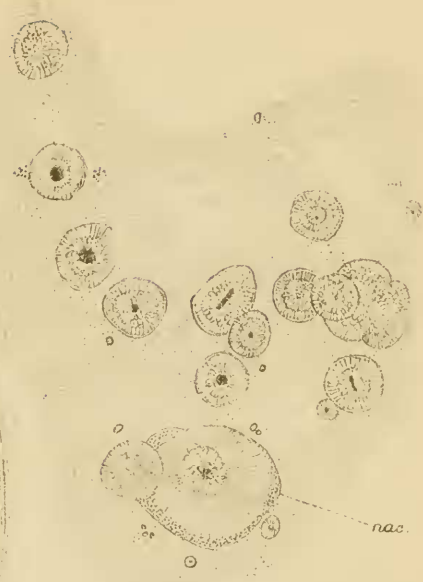
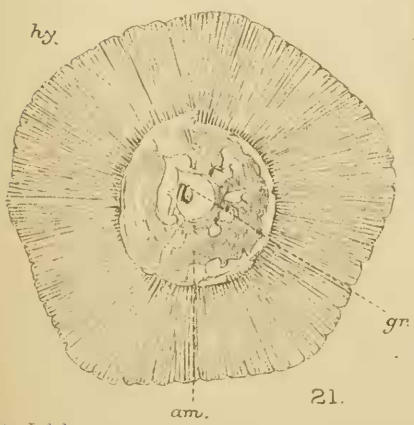
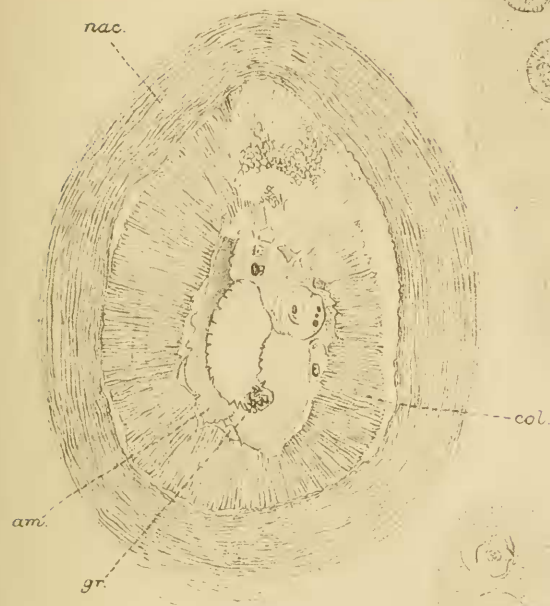


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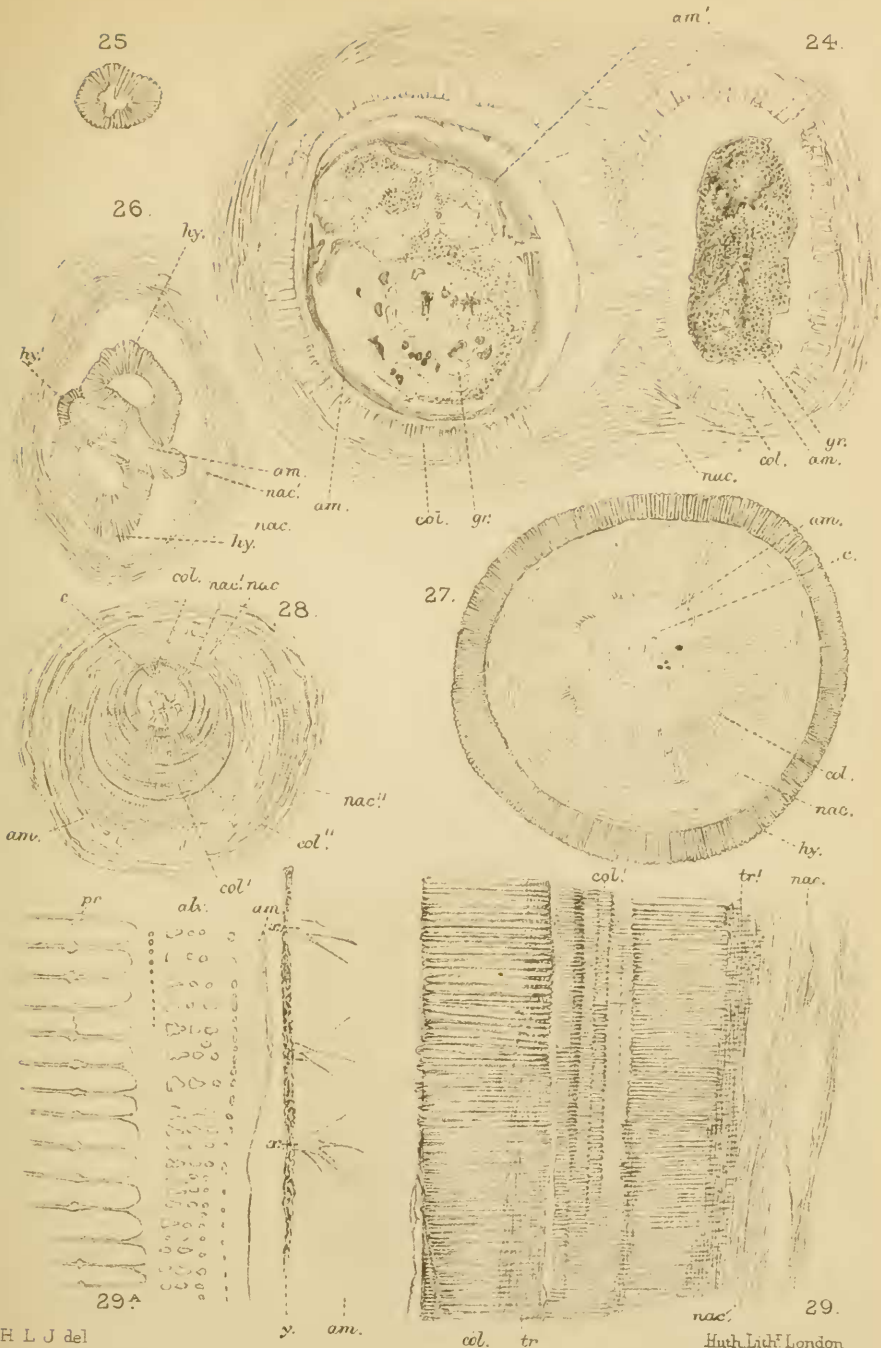
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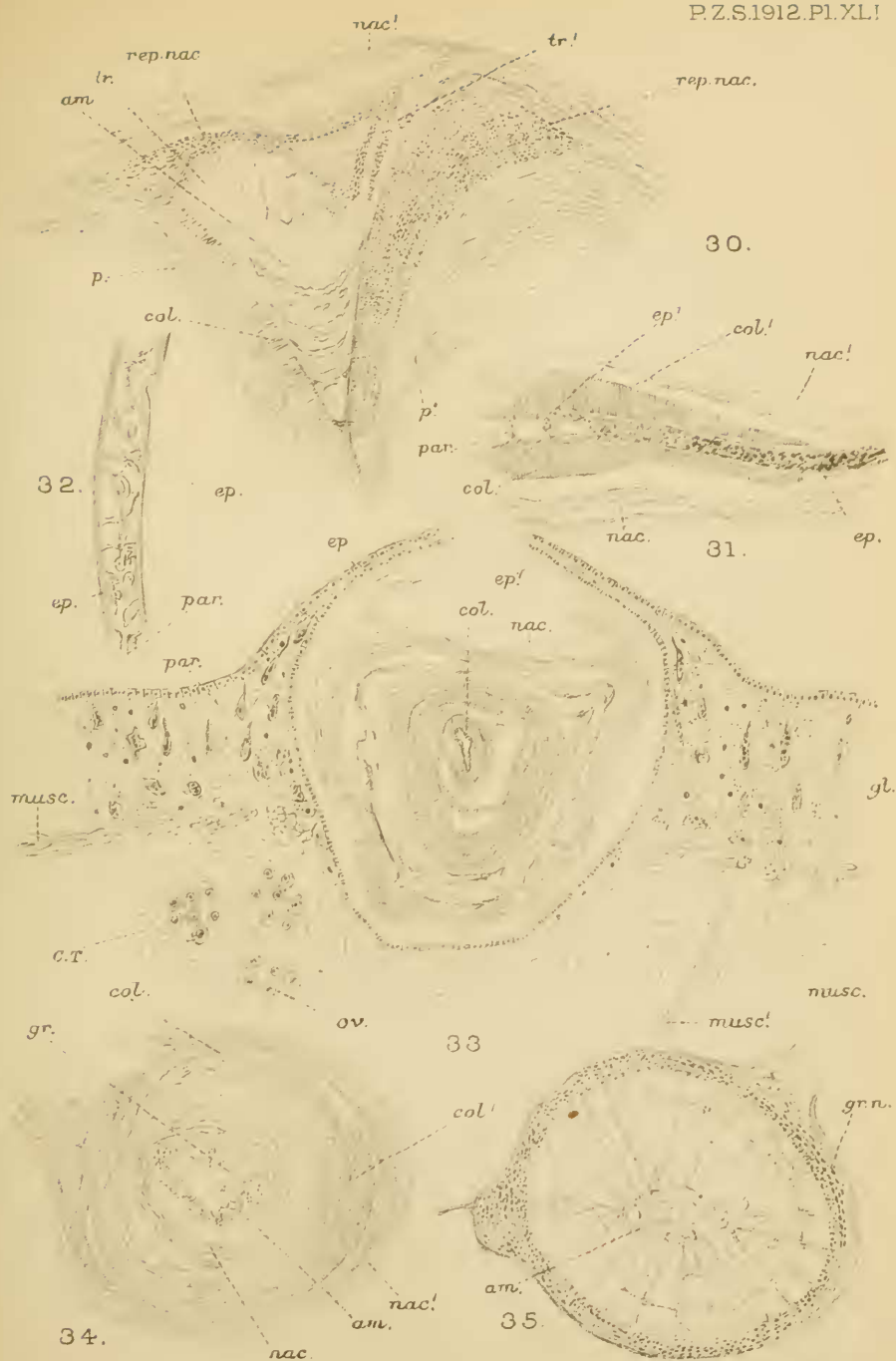


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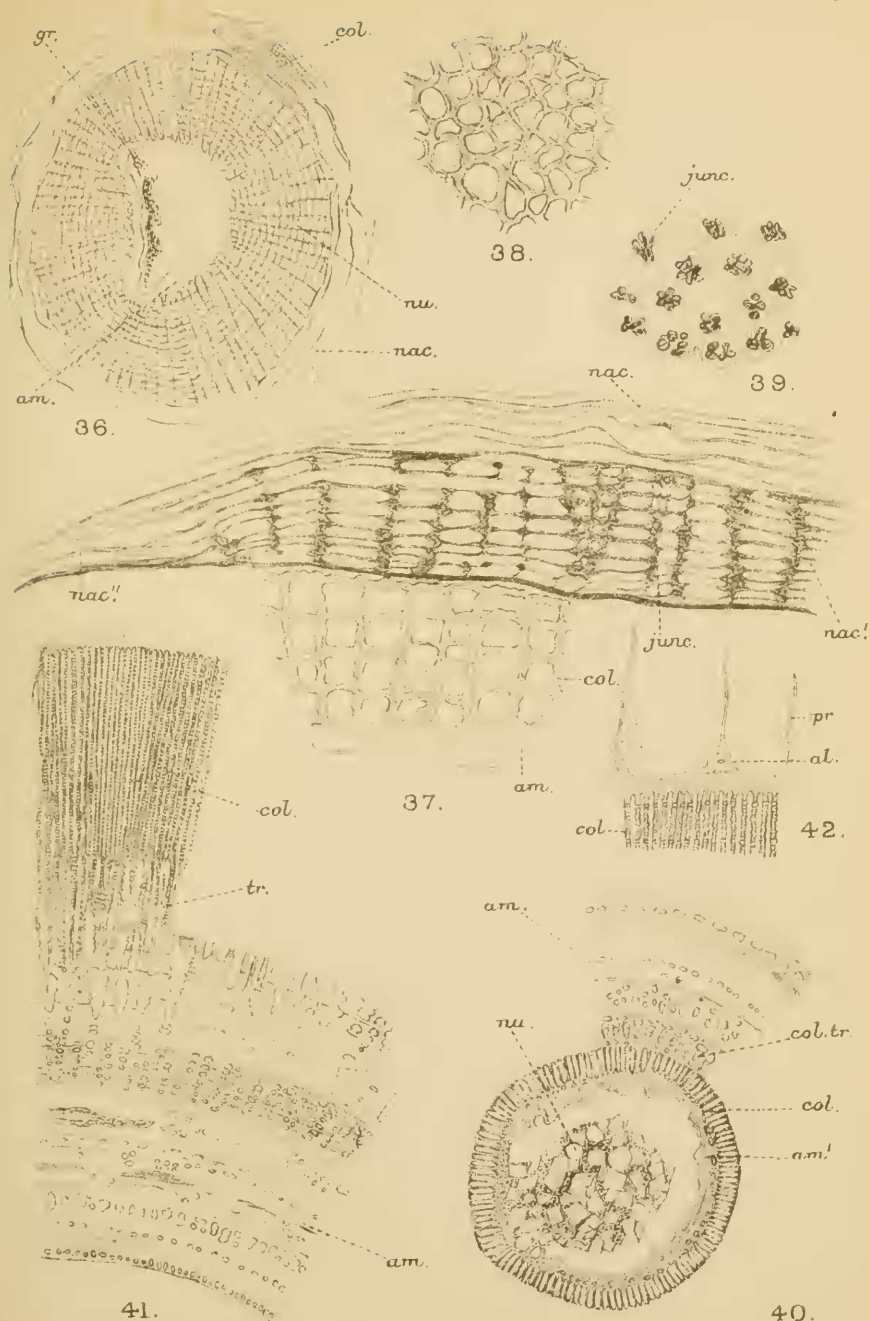


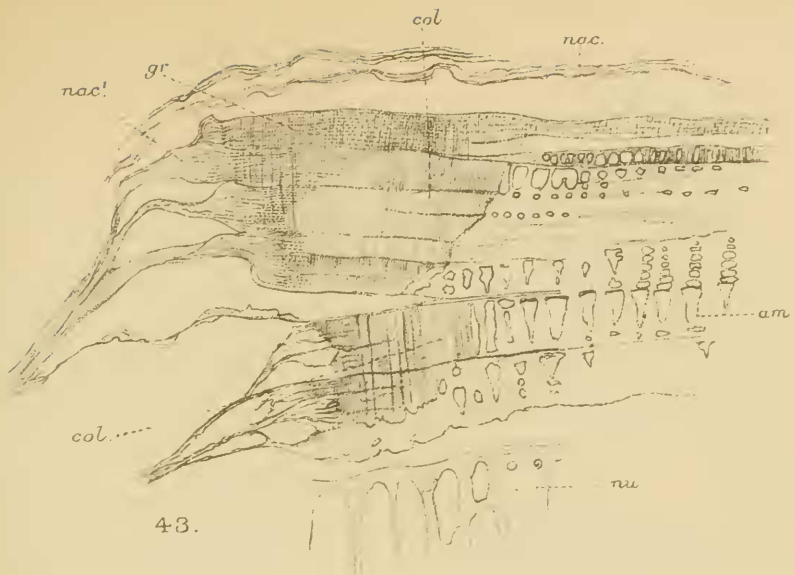


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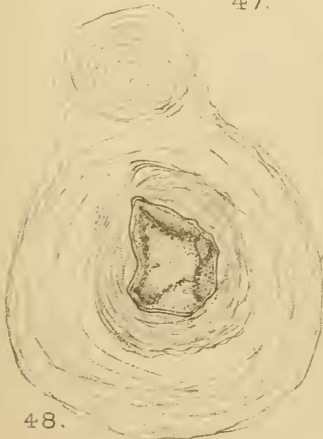
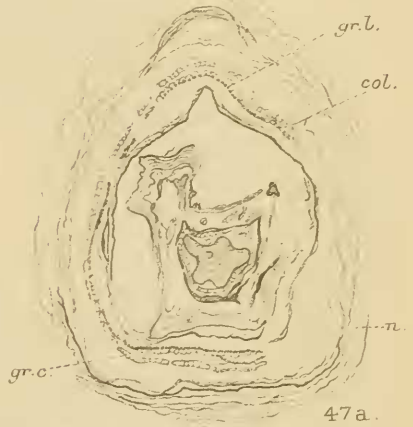
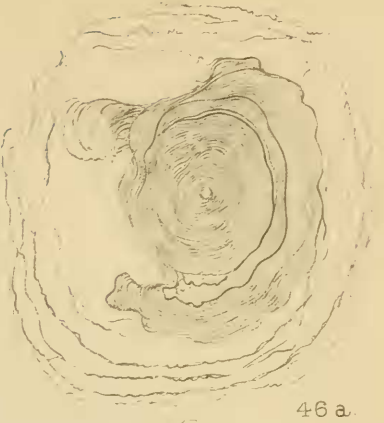
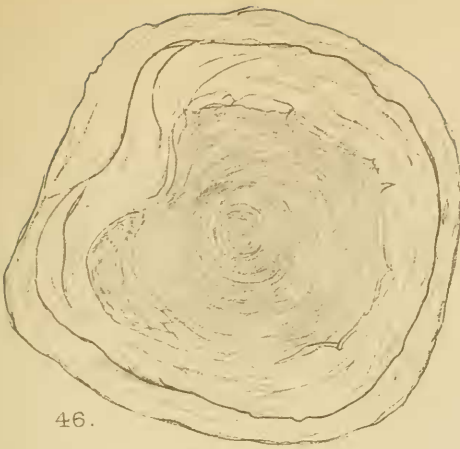


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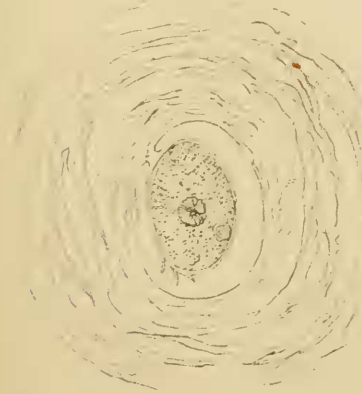
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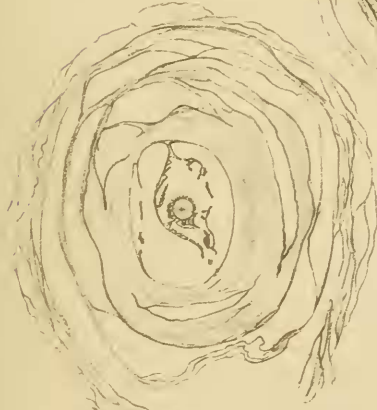


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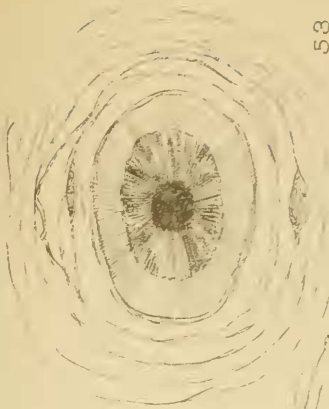
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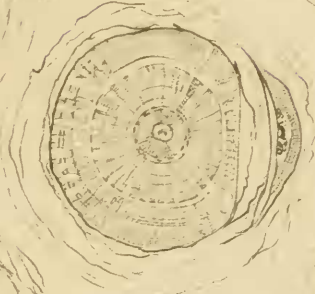
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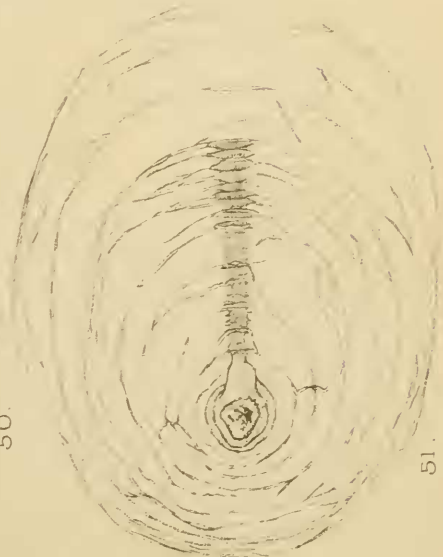
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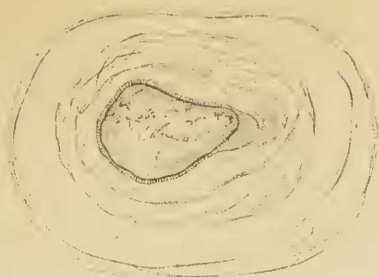


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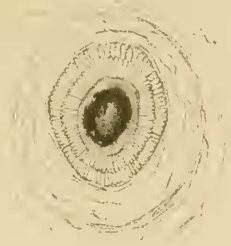
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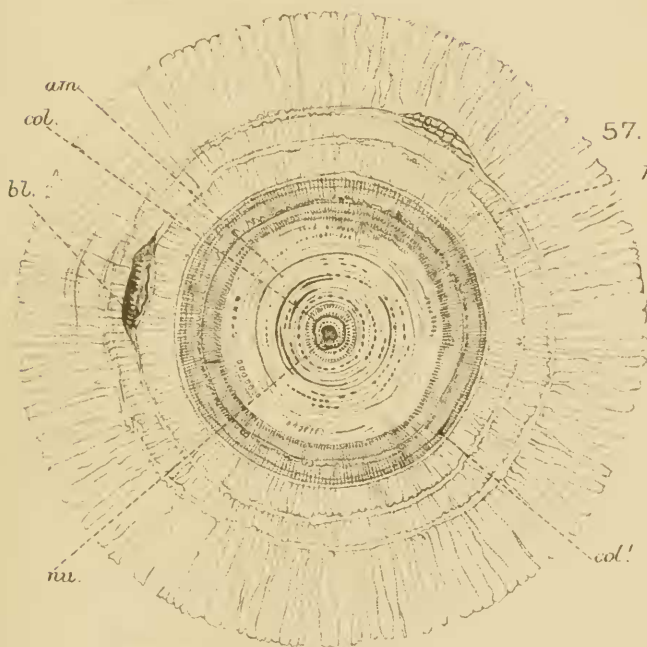
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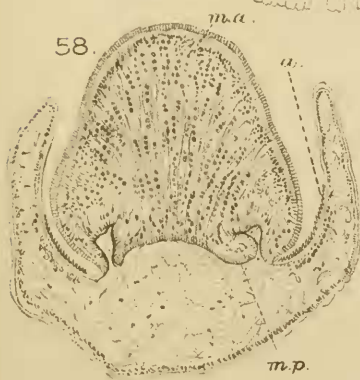
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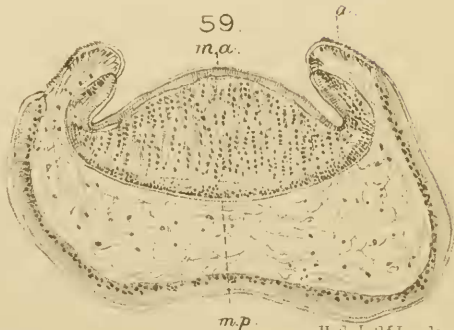
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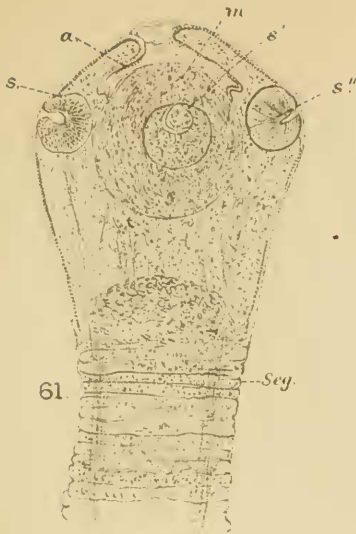


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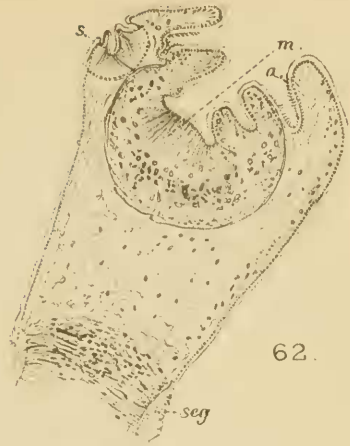
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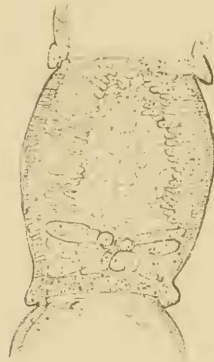
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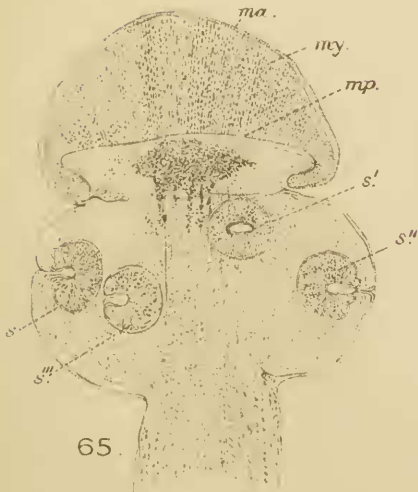
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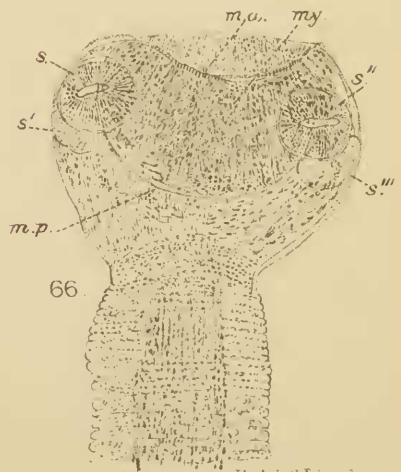
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on the Pearl-Oyster fisheries of the Gulf of Manaar (16) I was in South Africa, and material for the examination of some of his conclusions, which I was unable to harmonise with my own observations made prior to my departure from England, was not available.

Since my return I have made use of all available material—which is somewhat scanty; and while it is insufficient to enable me to propound, at the present moment, a working hypothesis as to the true cause or causes of the formation of Ceylon pearls, I think I have been able to show that the Cestode theory enunciated by Herdman, which has formed the basis of the somewhat meagre experiments which have been prosecuted in Ceylon since his return to England, and which even seems to have led to definite regulations elsewhere*, rests on quite insufficient evidence, and that, if the problem of the cause of Pearl-Production in *Margaritifera vulgaris* is to be solved, and a scheme for increasing the productivity of the Oysters evolved, a fresh start will have to be made.

The work that still remains to be done will centre around the causes which lead to the development of the epidermal sacs in which all pearls are formed—fine pearls and seed-pearls, “muscle”-pearls and “cyst”-pearls (or, as I prefer to call them, “parenchyma”-pearls), and the mechanism which controls the secretion of conchyolin and the deposition therein of carbonate of lime. In fact, I am led back to the principles enunciated by me in 1902 (25), that the essential element in pearl-formation is the pearl-sac, and not the nucleus, and that it is by a study of the causes which lead to the development of the former that the problem of the origin of pearls is to be solved.

Some material for these further investigations has just reached me as I write this introduction, and more is promised, but, owing to the difficulties and delays which may occur, I now publish my researches on the structure of the shell, and of pearls and their pseudo-nuclei and nuclei, without attempting to deal with the origin of the pearl-sac.

I take this opportunity of expressing my thanks to the Ceylon Company of Pearl Fishers, Ltd., for purchasing, on my behalf, pearls in Colombo to be used in these investigations; to Professor Raphael Dubois, for pearls from *Margaritifera vulgaris* from the Mediterranean; to Prof. W. R. Dunstan, F.R.S., for allowing me to make use of material in the Imperial Institute; to Mr. J. Calcott Gaskin, Assistant Political Agent at Bahrein, Persian Gulf, for sending me, in 1903, a number of preserved specimens of the Liugah shell (*M. vulgaris*), some of which contained pearls;

* Rules for Lower Burma under the Burma Fisheries Act, 1905. Rules 64 and 67, which prohibited the capture of *Balistes* and *Trygion* in the Pearl Fishery districts, and required them, if accidentally caught, to be returned to the sea, appear to have been inspired by the Cestode theory. These rules were cancelled in 1909. It may be remarked that the Pearl-Oyster of Burma (the Mergui shell of commerce) is not the same species as the Ceylon Pearl-Oyster, *M. vulgaris* Schumacher, but is the great Mother-of-Pearl Oyster, *M. maxima* Jameson.

to Mr. E. Hopkins, of Hatton Garden, for specimens of Ceylon Pearls; to Professor W. A. Herdman, F.R.S., for specimens of Pearl-Oysters from Ceylon, and for allowing me to examine his preparations; to Mr. Max Mayer, of Hatton Garden, for specimens of pearls for my work; to H.H. the Jam Sahib of Nawanagar, for specimens of Pearl-Oysters from the Gulf of Kutch; to Prof. L. G. Seurat, for pearls from New Caledonia, Madagascar, and the Gambier Archipelago; to Mr. A. E. Shipley, F.R.S., for allowing me to examine and make use of his specimens of *Tylocephala* and allied genera of Cestodes from Ceylon fishes; to Mr. E. A. Smith, I.S.O., for permitting me to make use of material in the British Museum (Natural History), including specimens from Dr. Kelaart's collection; and to Mr. A. Van Noorden (of the firm M. Myers, Mother-of-Pearl Merchants) for specimens of Lingah and other shells.

After this paper was handed in I received a copy of Rubbel's paper (34 a) setting forth in greater detail the results already published by him (33 and 34). Herr Rubbel and I have arrived, working independently on widely different molluscs, at identical conclusions on several important points, such as the nature of Herdman's "calcospherules." Where practicable, I have interpolated references to his work in the text, and my only regret is that it is not possible to discuss his valuable work more fully in the present paper and to dwell at length on the many points where, adopting a slightly different terminology and interpretation of the phenomena, our respective works lead to the same conclusions.

(2) OUTLINE OF THE RECENT INVESTIGATIONS IN CEYLON.

In January 1902 Professor W. A. Herdman went to Ceylon, at the request of the Colonial Office (who availed themselves of his services on the recommendations of the Council of the Royal Society and of Professor Ray Lankester), to investigate the condition of the Pearl-Banks. Professor Herdman took with him as his assistant Mr. James Hornell, who remained in Ceylon to carry on the work after the former's return to England in April of the same year, and who collaborated in the preparation of Professor Herdman's reports. Professor Herdman's visit in 1902 gave him seventy-eight days in Ceylon, and was largely spent in an extensive biological and faunistic survey of the pearl-banks, carried out on two successive dredging-cruises, each of several weeks' duration; and he credits Mr. Hornell with the major part of the observations on Pearl-production (Royal Institution Lecture of March 27th, 1903) (14). Prof. Herdman himself always seems to have regarded the condition and welfare of the natural beds of oysters as a more important problem than the question of pearl-production (Report on the Ceylon Pearl Fisheries, Part I. p. 5, and Part V. p. 29; also Report of the Annual Meeting of the Ceylon Company of Pearl Fishers, Ltd.,

for 1908*). In fact, he contends on p. 30 of Part V. of his Report, and in his address to the Linnean Society on 24th May, 1906, that

“to reverse the popular saying, if we attend to the prosperity of the bed as a whole, the individual oysters may be left to take care of themselves, both in regard to health and pearl-production.”

In January 1904 Mr. Hornell was appointed Marine Biologist to the Government, to which post were subsequently added the administrative duties of Inspector of Pearl-Banks. While holding these Government appointments Mr. Hornell continued to collaborate with Prof. Herdman, though it is clear that the executive and administrative duties attached to his post interfered not a little with the more strictly scientific observations. Thus, in his Report on the Inspection of the Ceylon Pearl-Banks, November 1905, Mr. Hornell says (23), p. 6 :—

“The working out of this material must of necessity await the long deferred time when a pause shall occur in the field work in which I have been engaged for the past eighteen months, and which permits me no leisure for the correlation and marshalling of biological data.”

And, again, in Reports from the Ceylon Marine Biological Laboratory, No. 1, p. 23, 1905, he says :—

“The Marine Biologist should be given opportunity to further investigate the life of the spherical Cestode so abundant in the Pearl-Oyster, and which is the inducing agency in the formation of ‘cyst-pearls’ (‘fine pearls’). The problem is far from solution, and will entail much unpleasant and trying labour before a satisfactory conclusion can be hoped for.”

The observations of Prof. Herdman and Mr. Hornell on the spot were corrected and correlated by laboratory work in Liverpool, carried out by Prof. Herdman and his staff at the University, on the material sent home for investigation. Prof. Herdman has courteously allowed me to examine the slides made during these investigations, showing sections of Pearls *in situ* in the tissues, and of the Cestode larvæ which he associates with pearl-formation.

In March 1906 the Ceylon Company of Pearl Fishers, Ltd., was formed and the pearl-fisheries were leased to the Company by the Government, at a yearly rental of Rs. 310,000, the lease carrying the obligation to spend, in addition to the above rent, a sum of from Rs. 50,000 to Rs. 150,000 yearly “on the experimental or

* Financial Times, Dec. 19, 1908. Sir West Ridgeway, Chairman of the Company, on this occasion said that

“with regard to biological research, Prof. Herdman was of opinion that in the present condition of the Company’s pearl-banks accurate navigation, careful and exhaustive inspection of the ground, and wise administration are more important than the purely scientific side of the business.”

practical culture of the pearl-oyster and on the improvement of the pearl-banks" (50).

On the formation of the Company Mr. Hornell was transferred to its service as local General Manager, Prof. Herdman being made Scientific Adviser.

In April 1908 Prof. Herdman, at the request of the Company, paid another short visit to Ceylon, to enquire into the question of the inspection of the banks and other branches of the business. As a result of Prof. Herdman's inquiries, the post of General Manager was abolished, being merged in that of Managing Director, and Mr. Hornell resigned, being succeeded by Mr. T. Southwell, A.R.C.Sc. (Lond.), who since 1907 had been acting as Mr. Hornell's assistant, and previously to that had assisted Prof. Herdman in his laboratory at Liverpool in the preparation of the material sent home for investigation. Mr. Southwell was made Scientific Adviser, a post which he still holds. Professor Herdman continued to be retained in an advisory capacity. Capt. J. Kerkham was appointed Superintendent of Fisheries*.

Besides the work of the Company's scientific employees, Dr. A. Willey, in his capacity as Marine Biologist to the Government (a post which he held along with the Directorship of the Ceylon Museum), has published some observations in the Ceylon Administrative reports and in '*Spolia Zeylanica*.'

Particulars of the work done, and of the conclusions arrived at, by these several naturalists will be given in the course of the paper.

In considering the incompleteness of the observations, despite the eight and a half years that have been devoted to the study of the Ceylon pearl-banks and the very large sums of money that have been expended, it must, of course, be borne in mind that for the last three or four years the banks are stated to have been practically bare of oysters †, and the prosecution of the investigations initiated by Prof. Herdman has thus been seriously hampered. But it is amazing that a Company whose prospects were so largely dependent on scientific work should have failed to set by an adequate stock of properly preserved material for scientific investigations and to establish at a suitable station a reserve of live oysters when the oysters were passing through their hands by the million. Had this been done, the barren years that have now come might have been devoted to the examination and amplification of Prof. Herdman's observations,

* Since the above was written the operations of the Company have ceased. It was announced in the '*Times*' of April 4th, 1912, that the lease had been terminated, a deposit of £10,000 together with the property of the Company being forfeited to the Government. An examination of the causes of the failure of this short-lived Company, which started with a capital of £165,000, has lately been published by the present writer (26 a).

† Not entirely; for it was possible to obtain 12,000 oysters in Feb. 1910 for Mr. Southwell's feeding experiment described in Part V. of the Ceylon Marine Biological Reports, p. 213, and no less than 35,000 oysters ranging from 8 months to 2½ years old were obtained for the experiment described in Part IV. of the same publication, p. 169. Mr. Southwell, in a paper published in May 1911 (42), says: "The only bed which now exists is confined to an inshore area, and the oysters found thereon only rarely contain the pearl-inducing parasite."

which of necessity were somewhat cursory and superficial. The result of this lack of foresight has been that the energy that ought to have been concentrated on an intensive study of the pearl-oyster and the mechanism of pearl-formation appears to have been largely dissipated on general faunistic work, such as the description of new species of crabs and tapeworms, matters which, valuable as they are from the purely scientific standpoint, have only a secondary bearing on the problem of increasing and rendering more reliable the supply of pearl-oysters and pearls.

(3) PROFESSOR HERDMAN'S CONCLUSIONS ON PEARL-FORMATION.

Professor Herdman distinguishes several causes of pearl-formation, though only two of these are regarded as of sufficient frequency to have economic importance, viz. Cestodes, causing the majority of "cyst-pearls," and "calcospherules," causing "muscle-pearls." I will pass over the pearly excrescences or "blisters" on the inside of the shell, due to the irritation of boring animals or intruding particles of foreign matter, as these should be kept in a category entirely distinct from true pearls. The latter term, following my paper published in 1902 (25), I shall confine strictly to bodies developed independently of the shell, which are not in any way continuous with the shell, except where, owing to the rupture or absorption of the intervening tissues, they may become secondarily covered over with nacre continuous with the lining of the shell. When this happens to a pearl it becomes an "attached pearl," a body quite other than a blister. Attached pearls are valued for the true pearl that can often be dissected out of them, whereas blisters are used as substitutes for pearls where the imperfect side can be concealed in the setting, *e. g.* in cheap jewellery, rings, pins, brooches, etc. Prof. Herdman (Report I. p. 10) apparently applies the name "ampullar pearls" to blisters, that is to say to bodies "which are not formed within closed epithelial sacs like the others, but lie in pockets or ampullæ of the epidermis," and on p. 146 of the same part speaks of blisters as "pearls of an inferior quality," but I cannot help feeling that, in scientific terminology at least, it is undesirable to apply the term "pearl" to these bodies at all.

Professor Herdman recognises the following causes of pearl-formation in the Ceylon pearl-oyster:—

(i.) *Grains of Sand and other Foreign Particles.*

These, in the experience of Professor Herdman and Mr. Hornell, only form the nuclei of pearls under exceptional circumstances. In the whole of their observations they have only records of three such cases out of hundreds of pearls examined (Report V. pp. 4 & 127). They say (V. p. 28):—

"Probably it is only when the shell is injured, *e. g.*, by the breaking of the 'ears,' thus enabling sand to get into the interior, that such particles supply the irritation that gives

rise to pearl-formation. The ectoderm, in such cases, would probably also be damaged, and cells may be carried in with the inorganic particles."

As shown below, the presence of grains of sand and other foreign particles in the nuclei of some Ceylon pearls has been confirmed by the present writer.

(ii.) *Boring Animals.*

While recognising that pearly excrescences or "blisters" are mainly due to borers such as *Leucodora* and *Clione*, Herdman and Hornell say (Report V. p. 28) that

"in exceptional cases a free pearl may be formed in this way."

No specific instances, however, are cited, nor is any explanation suggested as to what would be the mechanism in such cases.

(iii.) *Parasites other than Cestodes.*

In his lecture at the Royal Institution, referred to above, Prof. Herdman said:

"We shall I think be able to show in our final report that Cestodes, Trematodes and Nematodes are all concerned in pearl-formation."

At the same time he recognised the "larval Cestode of the *Tetrarhynchus* form" as the most important cause. Again, in the Report (V. p. 29), Herdman and Hornell say:

"A fuller experience is causing us to incline to the view that various parasites may act as pearl nuclei, even in the same mollusc. Some pearls are certainly formed around intrusive Nematodes. We have a complete cyst pearl, free and unattached, of which the nucleus is a coiled *Cheiracanthus uncinatus*, on which the pearl deposit is not sufficiently thick and opaque to obscure the coils so as to render identification difficult."

This pearl does not appear to be in Prof. Herdman's collection.

(iv.) *Pearls without a Nucleus.*

Prof. Herdman points out that both in the case of the Ceylon Pearl-Oyster and *Mytilus* some pearls have no trace of a nucleus (Report V. p. 18). He figures one such pearl from *Mytilus*, magnified 100 times. The existence of pearls without nuclei was recorded by Harley (11) in 1889. The observations set out below show that in the Ceylon Pearl-Oyster, both in muscle-pearls and in a great number of parenchyma-pearls, the presence of a nucleus of foreign origin is quite unnecessary, and point to the conclusion that the origin of the pearl-sac is usually due to stimulation other than that caused mechanically by such bodies.

So far as *Mytilus* is concerned, I attribute the conditions where

a nucleus is absent to two alternative causes. In the first case, the Trematode described in my paper on the Origin of Pearls in the P. Z. S. for 1902 (25) may migrate out of the sac, in which a pearl without any nucleus, or with a nucleus consisting of a few residual granules, may subsequently be formed. In the second case, in certain localities, *Mytilus edulis* produces pearls through an agency (which I am at present trying to investigate) other than Vermian. These pearls may have as nuclei either a few dark granules or nothing that can be detected without the use of much higher magnification than 100 diameters. One of them is shown at C in text-figure 33 (p. 277).

(v.) *Muscle-Pearls.*

The discovery of "muscle-pearls" was, I believe, first announced at the British Association Meeting in September 1903.

Under this name (Brit. Assoc. Report, Southport, 1903, p. 695) Prof. Herdman distinguishes pearls formed "around minute calcareous concretions, the 'calcospherules,' which are produced in the tissues and form centres of irritation." They occur "most abundantly in the muscular tissue near the insertions of the levator and pallial muscles." (Report, Part V. p. 27.) Herdman and Hornell say, speaking of muscle-pearls:

"it seems probable that these have been formed by the deposition of calcareous matter around a minute calculus in the tissues" "The Muscle pearls when present are usually abundant, and when examining under the microscope a young pearl of this kind, *in situ*, it is common to find a large number of minute calcareous depositions or calcospherules scattered in the neighbouring tissue. It is probable that the muscle pearls are formed around these microscopic calcospherules as centres of irritation, and as these [? their] positions are invariably in our experience close to the surface of the muscle or the mantle, there is no difficulty in understanding that there, if anywhere, ectoderm cells might migrate to the source of irritation and thus be responsible for the deposition of a pearl." (Report V. p. 27.)

Muscle-pearls are especially numerous in certain localities; Prof. Herdman instances (Report V. pp. 30-31) that they were particularly numerous on the S.E. Cheval Paar in 1902 and 1903, and

"that the vigorous and healthy oysters of the Eastern Cheval and Periya Paar Karai produce practically all the examples of this class of pearls,"

the numbers produced on other banks being insignificant.

Mr. Hornell, speaking of an examination of a number of pearls attached to the shell, states that "decalcification of the pearls attached to muscle-scars reveals no organic nuclei, whereas the [attached] pearls irregularly disposed have Cestode embryos as nuclei, exactly as 'fine' pearls have." (19, p. 12.)

Mr. Southwell (40, p. 194), referring to the calcospherules causing "muscle-pearls," says:

"The origin of the latter bodies is quite unknown, although it seems almost certain that they are depositions from the blood."

Again, in a later paper (42, p. 128), Mr. Southwell says:

"Other pearls are also found in the Oyster, but they have no organic nucleus. Such pearls are termed muscle or seed pearls. Their origin is obscure, but they are always found near the muscle insertions, and are believed to be formed round a calcospherule of excretory origin, or by the sheer of muscles moving in different planes."

In the first mentioned of the above papers (40), he goes on to say:

"Considerably more pearls are formed round calcospherules than round parasites, the ratio being about 13 to 1. They are therefore of considerable commercial importance."

Unfortunately, Mr. Southwell does not give the number or sources of the pearls on which this statement is based, although it is quite clear, from the observations of Prof. Herdman and Mr. Hornell, that "muscle-pearls" are characteristic of certain localities, and "cyst-pearls" of others, and that the ratio may vary greatly on different banks.

My own observations on "Muscle-pearls" and on the nature of the so-called "calcospherules" are given in a later part of this paper.

(vi.) *Cestode Larvæ.*

Of fine or "Orient" pearls Prof. Herdman and Mr. Hornell claimed that the most frequent nucleus is a Cestode larva. In their "Conclusions on Pearl-Formation" (Report V. p. 29) they maintain that their investigations have shown "that in *Margaritifera vulgaris*, at Ceylon, the production of the 'Orient' pearl is dependent upon Cestode infection and that the species mainly concerned is *Tetrarhynchus unionifactor*," and in the General Summary of their Ceylon Report (V. p. 127) they say:

"The majority of these fine pearls contain as their nuclei the more or less easily recognisable remains of certain Platyhelminthian parasites, which we identify as the larval condition of Cestodes belonging to the genus *Tetrarhynchus*."

It is stated several times that this supposed identification was made during Professor Herdman's second cruise in March 1902, but its elaboration must have been, in great part, the work of Mr. Hornell at a later date. The narrative of the Cruise (Report I. p. 70), published in 1903, states, in a paragraph apparently inserted between the records of March 6th and 7th:

"In the intervals of dredging and when moving from place to place, we were now continuously engaged in

examining the parasites of the pearl-oyster and their influence on pearl-formation. We also decalcified such pearls as were found. This work was continued as time permitted during the next few weeks, and also by Mr. Hornell after I left. We found various parasites, in the liver especially, some of which were Platyhelminthian and others Sporozoan in their nature, and some of which were enclosed in calcareous capsules. Mr. Hornell afterwards determined that these were *Tetrarhynchus* larvæ of Cestodes, and we have no doubt that they are in many cases the nucleus of the pearl, and the irritating cause of its formation."

Again, in the preface to Part II. of his Report, p. vi, dated July 1904, Prof. Herdman says :

"On the Cheval Paar, in March 1902, we satisfied ourselves that the 'Orient' pearl, free in the tissues of the pearl-oyster, is deposited around a cyst containing a Cestode larva, and preliminary notices to this effect were published in my Royal Institution Lecture of March 27, 1903, and at the Southport Meeting of the British Association in September 1903."

On p. 6 of Part V. of the Report (Pearl-Production), Herdman and Hornell say :

"One of the first facts that we were able to determine in connection with the Ceylon Pearl-Oyster, in the spring of 1902, was that the Orient pearl in the Gulf of Manaar is deposited around the young larva of a Cestode."

And on p. 15 :

"We found the Cestode larvæ in association with pearls in the tissues during our cruises in the 'Lady Havelock' in the Gulf of Manaar, in February and March, 1902. It was about March 6th (see Narrative, p. 70, in Part I.), when cutting up Oysters from the western part of the Cheval Paar, that we first became convinced that the opaque white globular larvæ we were finding encysted in the liver belonged to Cestode worms."

On the other hand, Shipley and Hornell, in their paper on the Parasites of the Pearl-Oyster (Herdman's Report, II.), seem to imply that at least the elaboration of these observations was carried out subsequently to Professor Herdman's departure from Ceylon. Thus, they say (p. 79) :

"These larvæ first attracted attention during the second cruise of the 'Lady Havelock,' on March 6th, 1902, when numbers of the early globular stage were dissected out from the livers of oysters dredged from the West Cheval Paar. Subsequently, during the investigation carried out at the Galle Biological Laboratory, a second and more advanced stage of a *Tetrarhynchus* larva was found in the same

material. Details of the morphology and histology were then worked out, and the relationship which the larvæ bear to pearl-formation was investigated."

Strange to say, Professor Herdman's Preliminary Report to the Government, dated July 1st, 1902 (13), makes absolutely no reference to the discovery in the previous March of this important aspect of the parasites of the Pearl-Oyster.

In the Preliminary Report referred to, Prof. Herdman says (p. 2):

"Samples of all the oysters obtained by us were examined for parasites and for any diseases or abnormal conditions, and although a considerable number of minute parasites, both Protozoan and Vermean, were found, still that is by no means unusual amongst molluscs, and we do not consider that we saw anything which gave evidence of any epidemic disease or widespread and injurious prevalence of parasites."

And again in the same Report (p. 4), in his summary of conclusions, the Professor says:

"A considerable number of parasites, both external and internal, both Protozoan and Vermean, were met with, but that is not unusual in molluscs, and *we do not regard it as affecting seriously the oyster population.*" (The italics are mine.)

In view of the last three quotations, if it were not for the very definite assertions in Part II. p. vi, and Part V. p. 6, of Professor Herdman's full report, quoted above, I should be almost inclined to think that, while the Cestode larvæ were no doubt discovered "in association with pearls" during Professor Herdman's cruise, the Cestode theory of Pearl-formation might have been evolved after Professor Herdman's return to England, and after the above-mentioned preliminary report had been submitted. In that case Mr. Hornell might well have been misled by the false analogy of the case of the Trematode origin of pearls in *Mytilus*, which was dealt with at length in my paper (25). This paper appeared in August 1902; that is to say shortly after the Professor's preliminary report of July 1st, 1902, containing no reference to the Cestode theory, was submitted. The view that my paper might have misled Mr. Hornell and Professor Herdman would also derive support from the fact that the real point of my paper had apparently been missed, viz. that it is not the presence of any parasite, but the *specific stimulation of a particular kind of parasite* that causes the growth of the pearl-sac. I consider this point is by far the most important contribution I have so far made to the subject, and I believe it will be the basis upon which a rational system of artificial pearl-production will ultimately be built.

The first announcement of Prof. Herdman's theory of Pearl-formation seems to have been made at a lecture delivered before the Royal Institution on March 27th, 1903, an abstract of which appeared in 'Nature' for April 30th of the same year (14).

(4) EXAMINATION OF THE CESTODE THEORY OF PEARL-PRODUCTION.

It is unfortunate that more figures of pearls containing as nuclei supposed Cestodes are not given in Prof. Herdman's account of Pearl-formation. The only figures that represent the nuclei of decalcified pearls examined entire* as transparent objects appear to be those on plate ii. in the Section on Pearl-Production in Part V. of the Report, figures 5 and 7, figure 6 representing a dead Cestode in a partially calcified cyst (not, however, a pearl). On p. 22 it is stated that these drawings, which are reproduced from Shipley and Hornell's article upon the parasites of the Pearl-Oyster in Part II. of the Report, are the work of Mr. Hornell, and it is not evident from the text that Prof. Herdman had ever seen the specimens from which they were made. Turning to these same figures on plate i. of the article by Shipley and Hornell on the parasites of the Pearl-Oyster (Part II. of the Report, figs. 5 (A) and 8 (B), (C), (D)), we find them described in the Explanation of the Plates as the nuclei of decalcified pearls; but the same figures are referred to in the text, p. 80, as representing the Cestode larva enveloped in its "tough elastic and fibrous capsule of spherical form, derived from the adjacent connective tissue cells."

It is, I think, hazardous to identify these figures as the remains of Cestode larvæ without examination of sections, and I cannot help feeling that each of these figures is capable of comparison with the non-Cestodian centres of pearls described by me below.

It is a remarkable fact that nowhere throughout the Report is there figured a *section* of a decalcified pearl showing the Cestode remains in the nucleus, and to this fact I may add my own observation that of all the pearls sectioned *in situ* by Prof. Herdman, numbering about 25 (not counting some minute clustered muscle-pearls), which he kindly sent me to examine, I could not find a single nucleus that I was able to accept as being a Cestode or other Vermian parasite. The characters of the nuclei in these preparations are described in the part of the paper which deals with my own researches.

The superficial resemblance of the pearl figured on plate ii. fig. 4 A, in Part II. (Parasites) of the Report, and again in Part V. (Pearl-Production), pl. i. fig. 5 *k*, *c*, *d*, & *e*, to the globular Cestode larvæ found in the Oyster is hardly enough to go upon. If such a pearl consisted of a parasite thinly coated with nacre it would probably be dark and valueless and not a "fine pearl," for the yellowish-brown dead tissue of the Cestode should be clearly visible through the nacreous coat. It is not stated whether this pearl was decalcified and sectioned to test whether the resemblance was more than "skin deep."

* These figures are referred to by Southwell (42), p. 128, as "figures of sections of decalcified pearls," but they are not described as such in the text; and they certainly appear to be no more than drawings of the centres of pearls examined as transparent objects.

With regard to the mechanism by which the Cestode is supposed to cause pearl-formation, Prof. Herdman is unable to contribute much. He seems to recognise that the particular conditions necessary to transform the Cestode into a pearl-nucleus are not by any means universally present, and that it is only, so to speak, under exceptional circumstances that the Cestode, which is very abundant in the Ceylon Pearl-Oyster, becomes the centre of a pearl. The larva is surrounded by a connective-tissue cyst, and has not been satisfactorily demonstrated in any instance with an epithelial "pearl-sac" (such as I described for the Pearl-inducing Trematode in *Mytilus*), though supposed proliferations of cells inside the connective-tissue cyst are figured in the Report (Part V. Pearl-Production, pl. iii. fig. 7). These, being inside a thick fibrous connective-tissue capsule, are difficult to accept as being equivalent to a pearl-sac, which I generally find to be surrounded by the spongy subepidermal parenchymatous tissue, except in the case of those parts of a "muscle-pearl" into which muscle-fibres are inserted. From my own observations I am rather inclined to regard these "cells" as granules excreted by the parasite itself, with possibly an admixture of wandering leucocytes. In any case, if this is an epithelial pearl-sac, what becomes of the thick fibrous cyst outside it, which is certainly not present around the pearls? Professor Herdman himself (see below) does not think the Cestodes enveloped in thick connective-tissue cysts are destined to become nuclei of pearls.

The supposed migration of ectoderm-cells into the wall of a pearl-sac already formed and already containing a pearl, as figured in Part V. (Pearl-Production), pl. i. figs. 18-20, seems to be a matter quite apart from the question of the primary origin of the pearl-sac.

On p. 23 of Part V. Prof. Herdman says:—

"It is quite evident from the examination of a large series of sections, such as we have worked through, that the majority of these encysted parasites do not become encased in pearls. Probably none of those in thick connective-tissue cysts are destined to form nuclei. They are awaiting their legitimate further development in the next host, after their sheltering mollusc has been devoured by a fish. In such cysts and around such parasites we find no epithelial sac, and, as a consequence, there can be no pearl. Whether or not it is the case that only dead parasites supply the stimulus necessary to induce pearl-formation, and whether, as Giard has suggested, the parasites may be infested and killed by a species of *Glugea*, so that that Sporozoan comes to be eventually responsible for the pearl, we are not prepared to say—we have found no fresh evidence in the Ceylon material bearing upon that point. It seems clear to us, however, that the epithelium is always associated with pearl-formation, and that in the absence of the epithelium only a thick-walled connective-tissue cyst is produced. If we adopt the view (see

below) that this epithelium is genetically related to the ectoderm, then a possible explanation of the difference in behaviour in the encysted condition would be that those larvæ that carried in ectodermal cells become covered (when dead or while still alive) by a pearl-sac and embedded in a pearl, while those that were free from ectoderm become surrounded by the connective-tissue cyst."

No satisfactory instance, however, is recorded of the Cestode parasite being observed surrounded by an epidermal sac.

Again in Part III. of the Report, p. 32, Professor Herdman, quoting a Report furnished to him by Mr. Hornell, says the abundance or otherwise of cyst-pearls "is connected with the factors which control the relative abundance of the pearl-inducing Cestode and those which conduce to its death during encystment in suitable localities within the tissues—problems as yet obscure"; while in Part V. (Pearl-Production), p. 15, he says "it is apparently very difficult indeed to hit upon a stage showing the commencement of the pearl-formation."

And again, in an address delivered at the Anniversary Meeting of the Linnean Society of London in 1906 (17), Prof. Herdman, speaking of his Ceylon work, says that it is probably only those Cestodes that are provided with an ectodermal covering forming a pearl-sac that become the nuclei of pearls. But, as stated above, such an ectodermal pearl-sac has not yet been found to occur around the parasite, and its occurrence is purely theoretical.

The investigations made subsequently to the publication of Prof. Herdman's Reports have added little to our knowledge of the subject. In 1905 Mr. Hornell published a Report on the *Placuna placenta* pearl-fishery of Lake Tampalakamam, dated June 15th, 1905 (21). In this he states (p. 5) that he dissolved two *Placuna* pearls (out of five in his possession) and found that "in each case the nucleus proved to be the dead remains of a minute Platyhelminthian larva of the same stage and species as that which forms the nucleus of cyst-pearls in *Margaritifera vulgaris*." Further study revealed the presence of Cestode larvæ in the dorsal portion of the visceral mass. He considers that these are identical in details of form and structure with those of *Margaritifera vulgaris*, but expresses some doubt as to their specific identity, and adds that if they prove distinct the *Placuna* parasite will need a fresh name. Mr. Hornell further observed that these larvæ multiply asexually by a process of endogenous budding, which he wrongly refers to as parthenogenesis.

Mr. Hornell then goes on to make the following extraordinary statement, which I quote as giving some indication of the confusion of ideas which existed as to the identity of the supposed pearl-producing larvæ even in 1905, *i. e.* nearly three and a half years after the beginning of the observations:—

"The discovery of a stage in the life-history of this parasite, which I am confident is homologous with the
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Redia-stage of a Trematode, confirms my original idea of it being a larval Trematode—an idea formed when first I saw it in March 1902” [*i.e.* the Cestode larva in *Margaritifera vulgaris*]. “Other facts point to the same conclusion, and I have now no doubt on the subject. The genus to which it belongs is still doubtful, but as it is inconvenient not to have a name whereby to make mention of it, I shall henceforth refer to it under the cognomen of *Distomum* (?) *margaritifactor*, n. sp., the specific name having reference to the fact that it is the inducing cause in the production of ‘fine’ pearls.”

Mr. Hornell anticipated that asexual reproduction would be found to occur in the Cestode parasites of *Margaritifera vulgaris* also, and this has since proved to be the case.

An expurgated edition of the above report was published in 1906 (22), which, however, bore the same date, June 15, 1905, as the Sessional Paper. In this the statements as to the supposed Trematode nature of the parasite were suppressed (though not formally withdrawn), the name “*Distomum margaritifactor*, n. sp.” cancelled with a stroke, still figuring in the lithographed plate (Annexure II.).

As explained below (p. 345) I have been unable to confirm the presence of Cestodes in the centres of *Placuna* pearls from Ceylon, any more than I can find them in the pearls of *Margaritifera vulgaris*.

In 1907 Dr. A. Willey (43) confirmed and extended Mr. Hornell's observations on the endogenous reproduction of the *Placuna* Cestode.

Mr. Southwell discovered (39, p. 173) that endogenous asexual reproduction or budding, similar to that described by Hornell and Willey in the parasite of *Placuna*, occurs occasionally in the Cestode parasites of the Pearl-Oyster. He only observed the occurrence twice, in November 1906 and January 1909—in each case a single endogen was found.

In 1903 the late Professor A. Giard (10) announced that M. L. G. Seurat believed that in the black-lipped Pearl-Oyster of the Gambier Archipelago (*Margaritifera margaritifera* var. *cumingii* Reeve) pearl-formation was due to the presence of a parasite, figures of which were given, and which Prof. Giard referred to a genus near to *Cyathophyllus* [*Cyathocephalus*?] Kessl. or *Acrobothrium* Olsson.

Subsequently Seurat found the adult of this worm in the Eagle Ray (*Aetobatis narinari* Euphr.) in the spiral intestine, and named it *Tylocephalum margaritiferae* (36). The adult, which is figured by Seurat (37), is quite a minute worm, not exceeding 4 mm.

The scolex occurs in cysts, similar to those occurring in the Ceylon Pearl-Oyster, and multiple cysts (perhaps formed by budding of the larva as in the *Placuna* parasites) occur also. Seurat

states that these cysts which form around the parasites become the nuclei of pearls, and a decalcified pearl shows an organic nucleus in the centre surrounded by concentric layers of conchyolin, the whole having a diameter of about a millimetre, and the nucleus being a scolex .225 mm. long and easily recognisable as that of *Tylocephalum*.

It appears that in this case also the parasites may be present in great numbers without pearls being found (Seurat (35), 1904, p. 295).

Here, again, examination of the scanty material available (see p. 346) has yielded no confirmation of the presence of Cestodes in the pearls of *M. margaritifera* var. *cumingii*. I am endeavouring to obtain further material from the Eastern Pacific, in order to extend my observations on this species.

I may say that from the first time I read Professor Herdman's Reports and papers on the subject I was sceptical as to the relationship of the Cestode to pearl-formation.

Indeed, before Prof. Herdman's departure for Ceylon, on examining Dr. Kelaart's material at the British Museum, which Mr. E. A. Smith kindly placed at my disposal, I had detected the existence of these Cestode larvæ (which Kelaart seems to have regarded as "eggs of Entozoa") in their connective-tissue cysts in the Ceylon pearl-oyster, and after having examined the larvæ, and also having decalcified pearls from the same oyster, dismissed the Cestode as probably not concerned in pearl-formation.

My chief grounds for doubting the Cestode theory were the following:—

(a) The absence of evidence that the Cestode ever occurred in an epidermal sac, and the fact that it was almost invariably surrounded by a fibrous capsule or cyst which does not occur around the pearl.

(b) The large proportion of the Cestodes that showed no sign of becoming pearl-nuclei, pointing to the conclusion that pearl-formation does not necessarily, or even normally, follow from infection. Thus, in a footnote to p. 12 of Part V. of his Report, Prof. Herdman says:

"In comparing these statistics [*i. e.* of numbers of parasites and of pearls in *Mytilus*] with those of the Ceylon pearl-oyster, one is struck by the wholly different ratio borne by pearls to parasites in the two cases. In the mussels, pearls are far more numerous than the living parasites. In our Ceylon oyster, parasites may be exceedingly abundant*; while pearls (cyst-pearls) are relatively very rare, probably not more than one to a hundred parasites."

* Mr. Southwell (42), p. 128, says: "As many as 120 have been counted in a single oyster"; and further down on the same page: "Occasionally several hundred oysters can be examined, each containing 20 or 30 cysts, and not a single pearl is to be found."

In this connection an observation made by Dr. Willey (49) is very significant. Dr. Willey says:—

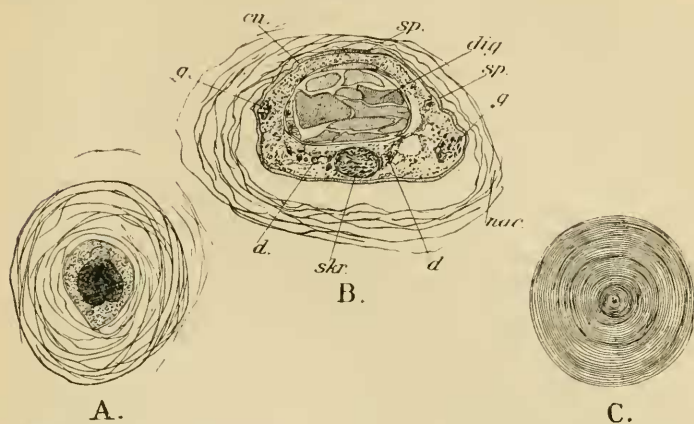
“A remarkable fact, indicating the subtle dependence of the pearl-producing molluscs upon their environment, is that whereas the most valuable pearls, called cyst-pearls by Prof. Herdman, are formed about a parasite as their centre or nucleus, yet the presence of these parasites in great numbers does not necessarily predetermine the formation of pearls. Pearl-oysters at Trincomalee may be heavily infected with the parasites without yielding pearls. It may be said that the parasitic infection and the pearl-disease are two separate phenomena, the latter proceeding from the former under certain conditions which are realised in the Gulf of Manaar. Whether these exact conditions can be reproduced elsewhere is one of the main problems before the Company. In the same way the cultivation of the oysters and the multiplication of pearls are two separate operations, the latter proceeding from the former in response to certain conditions affording the suitable stimulus. Conditions may favour the bivalves, but not their parasites; or they may favour both hosts and parasites, but not the production of pearls.”

(c) Professor Herdman's statement on p. 17 of Part V. of his Report that *Mytilus* pearls (which he examined in order to be able to correlate his work with mine) differed from Ceylon pearls in “the large size of the nucleus in the pearl (where a nucleus is present) and its characters, which are quite different from those of the encysted parasites in the Ceylon Pearl-Oyster.” Now the nucleus of a *Mytilus* pearl is generally about 0·5 mm. in diameter—the size of the Trematode when contracted into a sphere. As the Ceylon Cestode-parasite measures roughly from 0·5 to 1 mm. in diameter, the nucleus of a Ceylon pearl, if composed of its calcified remains, should if anything be larger, rather than smaller, than that of a *Mytilus* pearl. And the characters of the nucleus should not differ greatly—the dark opaque yellowish or brownish substance formed by the decomposition and subsequent calcification of the parenchymatous and muscular tissues of a Trematode should not differ materially in appearance from the analogous remains of a dead Cestode.

For purposes of comparison I figure below (text-fig. 33, A & B) a Trematode pearl from *Mytilus*, from Foulney, Lancashire (Preparation CIII). A shows the pearl decalcified and examined entire in oil of cloves, B shows the nucleus in section. In both cases the foreign nature of the nucleus is obvious, quite apart from the fact that in this preparation its Trematode character is quite clear (which would, of course, not be the case where decomposition had advanced considerably before calcification commenced). The characters of this nucleus are quite different from those of the pseudo-nuclei of Ceylon pearls figured in the

plates, the concentric stratification of the majority of which never occurs in a Trematode nucleus, and could hardly be expected in a Cestode.

Text-fig. 33.



A *Mytilus* pearl, from Foulney, near Piel, Lancashire, with a Trematode nucleus: A, examined entire in oil of cloves, after decalcification; B, the same in section. In A the oral sucker and digestive caeca of the worm are distinctly visible. In B the internal anatomy is still preserved. *cu.*, cuticle; *sp.*, spines on same; *dig.*, digestive caecum; *skr.*, ventral sucker; at *d* and *g* are dark masses, which may well represent the remains of the yolk-glands and gonads; *nac.*, nacre. C, a *Mytilus* pearl of non-Trematode origin, from Plymouth. Here the nucleus is, as in many Ceylon pearls, a minute group of granules. A $\times 20$; B $\times 70$; C $\times 20$.

As Mr. Cyril Crossland, Marine Biologist to the Sudan Government, is quoted by Professor Herdman (Report Ceylon Pearl Fisheries, Pt. V. Pearl-Production, p. 3) as supporting the Cestode theory, so far as *M. vulgaris* in the Red Sea is concerned, I wrote to him to ask him for further information. He replied, in a letter dated December 9th, 1911:—"I never published any statement that Cestode larvæ caused pearl-formation in the Red Sea. The evidence to my mind is in need of revision. In all cases the first result of excessive stimulation of the secretory epidermis of the mantle is the formation of a dark brown horny material [*i.e.* my amorphous substance.—H. L. J.]. How would this stain in sections, and is it cellular like the horny material of the prismatic layer? If so, would not a shrunken nucleus of such material resemble the dry remains of a Cestode? This is a criticism which I have had in mind several years, and have never put it to the test." From this it is clear that Mr. Crossland, though cut off from the possibility of applying modern laboratory technique, has arrived at much the same conclusion as that which I am elaborating in this paper.

To summarise the supposed relation between Cestodes and

Pearls, as described by Professor Herdman and Mr. Hornell, the position is briefly this:—

- (1) Ceylon Pearl-Oysters were found to contain large numbers of Cestode parasites which occurred simultaneously with pearls, but which did not necessarily result in the formation of pearls.
- (2) The nuclei of the majority of "cyst-pearls" were thought to be identified as consisting of the remains of these parasites, though Mr. Hornell's figures of such nuclei are capable of other interpretation.
- (3) No satisfactory evidence was adduced of the Cestode having acquired a surrounding epidermal sac, such as is normally formed around the pearl-producing Trematode in *Mytilus*, though Prof. Herdman admits that this sac is essential for pearl-production. The first stages in the supposed process are therefore purely hypothetical and unsupported by observation, besides pre-supposing an abnormal departure from the parasite's usual habit.

The evidence in favour of the theory is mainly that the more highly infected the oysters are with these particular Cestodes, the richer they are in pearls. Thus it was observed in 1904 (Report III. p. 32) that the oysters from the North-West Cheval, besides being the most extensively infected with Cestode-cysts were also the richest in cyst-pearls. And, again, Mr. Southwell records (40, p. 194), that

"the infection of the very old oysters [with tapeworm-cysts] found on the Kondatchi Paar in 1908 was remarkably low, and, as bearing practical proof that infection and pearl yield are intimately connected, it is interesting to note that the pearl yield also was remarkably low, the valuation only working out at about Rs. 18 per 1,000 oysters."

These facts might be explained, however, on the assumption that the conditions favourable to pearl-production are also favourable to Cestode infection.

Characters, Identity, and Life-Histories of the Cestode Parasites of Margaritifera vulgaris.

Apparently the first announcement of the supposed relation between the Cestode and pearl-production was made by Prof. Herdman at his Royal Institution lecture on March 27th, 1903 (14). In this he says that he and Mr. Hornell have proved so far "that in Ceylon the most important cause is a larval Cestode of the *Tettrarhynchus* form." Again, in the Report of the British Association, Southport, 1903, p. 695, Prof. Herdman says: "The parasite in the case of the majority of the cyst-pearls of Ceylon is the larva of a Cestode which appears to be new, and will be described under the name of '*Tettrarhynchus unionifactor*'"; and the pearl-inducing parasite is referred to throughout Prof. Herdman's Report under this name.

But the worm specifically described by Shipley and Hornell as *Tetrarhynchus unionifactor* on p. 88 of Part II. of Prof. Herdman's Report (Parasites of the Pearl-Oyster) and figured in plate ii. figs. 19 & 20, is a well-advanced *Tetrarhynchus* 6·5 to 7 mm. long, which occurs in and around the intestine of the Pearl-Oyster; and, to say the least of it, it is doubtful whether this worm is a later stage of the globular cysts, which Prof. Herdman identified as the nuclei of pearls, and not an entirely distinct organism. In order to avoid confusion of terms I am therefore giving separate names to the larger and smaller globular Cestode larvæ which Herdman recognises, as it is calculated to lead to much confusion of issues if these are referred to by the name of *Tetrarhynchus unionifactor* before their identity with it can be demonstrated more satisfactorily. The arguments for regarding the supposed pearl-producing parasites as distinct from *Tetrarhynchus unionifactor* are set forth below. In the absence of satisfactory evidence of their relation to the genus *Tetrarhynchus*, I propose, following Seurat (36), to whose larval Cestode, mentioned above, they bear a close resemblance, to refer them to the genus *Tylocephalum** and to describe the larger and smaller forms respectively as *Tylocephalum ludificans*, sp. n., and *Tylocephalum minus*, sp. n.

Two well-marked sizes occur in these globular larvæ and they are regarded by Herdman as distinct organisms (Report V. p. 21). On the other hand, Southwell considers that the asexual reproduction, which he has occasionally observed, accounts for the varying sizes of the larvæ in the Ceylon Pearl-Oyster, and says: "I am now convinced that these different sizes merely represent the same species in different grades of development." I am inclined to share Prof. Herdman's view that these two sizes are distinct organisms. It may even prove that there are more than two species represented. Indeed, I should not be surprised if further research on fresh material were to show that both *Tylocephalum ludificans* and *T. minus* are composite species.

Professor Herdman regards *T. ludificans* as the earlier stage of *Tetrarhynchus unionifactor*, and calls attention to its resemblance to Van Beneden's ideal figures of the young of *Tetrarhynchus*, while he treats *T. minus* as another species of *Tetrarhynchus* in its earlier stages. Nevertheless, he seems to have had suspicions that some, at least, of these larvæ might be *Tylocephala*, though he appears in the end to have decided that they—and presumably with them Seurat's larva—are a hitherto unknown stage in the life-history of the genus *Tetrarhynchus*.

In the Preface to Part II. of his Report he says (p. vi) :—

"It is possible that some of our Ceylon Pearl-Oyster parasites may also belong to the genus *Acrobothrium*" [*i. e.* the genus to which Seurat's larva was then referred], "although the more advanced ones are certainly Tetrarhynchids";

* Linton (27 a), pp. 805-9, pl. ix, figs. 5-9. Type *T. pingue*, from spiral valve of *Rhinoptera quadriloba*.

while in Part V. of the Report, p. 14, he and Hornell say:—

“Some of our Ceylon Pearl-Oyster parasites very closely resemble the figures given by Giard” [*i. e.* of Seurat’s larva] “and possibly may also belong to the genus *Cyathocephalus* [*Tylocephalum*], although most of them are certainly Tetrarhynchids”;

and on pp. 16-17:

“It is possible, however, that more than one species of Cestode is represented—one is certainly a species of *Tetrarhynchus* (*Rhynchobothrius*), and another is probably the same genus, or may possibly belong to *Cyathocephalus* . . .”

Later on, however (p. 20), Herdman and Hornell reject the idea that the globular larvæ may be *Tylocephala* or allied genera, and, in discussing the opinions of Giard and Seurat on the systematic position of Seurat’s larva, they say that they regard the terminal invagination, not as a sucker with a papilla on its floor, but as

“the opening in a hood or depression formed by the sinking of the scolex into the front of its vesicle. The changes of shape which we observed in this larva in the living state, the protrusion and retraction of the papilla-like part which we regard as the anterior end of the scolex, agree with this interpretation. Consequently, we are of opinion that this larval Cestode is not one of the Monobothria—that it belongs to neither the Pseudophyllidea nor the Tetraphyllidea, but is a young Tetrarhynchid belonging to the Trypanorhyncha, and we give here (fig. 4) a series of diagrams in order to show the positions that we suppose our stages to occupy in the development of such a form.”

Shipleigh and Hornell (Herdman’s Report II. p. 80) call attention to the resemblance of older examples of the larger larva (*Tylocephalum ludificans*) to Seurat’s form, and think there is little doubt that they are at least generically the same (p. 82). Again, Southwell says (39, p. 169):

“It would certainly appear more probable as well as simpler, for this larva to develop into a *Tylocephalum* (as is believed by Seurat) than into a *Tetrarhynchus*.”

Again, Southwell, speaking of the great scarcity of the adult of *Tetrarhynchus unionifactor* in Elasmobranchs taken by trawling, says (42 p. 130):

“It would almost appear that this fact in itself is sufficient proof that the adult of the pearl-inducing worm is not *Tetrarhynchus unionifactor*.”

But at the foot of the same page he reverts to the position that it is a *Tetrarhynchus*.

It is strange how the *Tetrarhynchus unionifactor* theory, once enunciated, has prevailed:—indeed nobody seems to have seriously followed up the obvious clue given by Seurat's discovery of the supposed adult of his worm.

Prof. Herdman says in his Report, Part V. pp. 20–21 :

“Shipley and Hornell in Part II. (p. 77) described and figured various stages of the Cestode larvæ both from the centre of decalcified pearls and also free in the tissues of the pearl-oyster, but left it an open question whether the sub-globular younger larvæ [i. e. *Tylocephalum ludificans*, n. sp.] belong to the same life-history as the elongated older forms, which are young *Tetrarhynchids*. If our arrangement of the stages observed in the tissues of the pearl-oyster is correct, and if all these larvæ belong to the same species, then the interpretation we have given above brings us to the conclusion that the larger of our two globular larvæ belongs to the worm which Shipley and Hornell described as *Tetrarhynchus unionifactor* in 1904.”

And, referring to some figures of *Tylocephalum ludificans* on pl. iii. (Report, Part V. Pearl-Production) figs. 1–8, he says (p. 21):

“there can scarcely be any doubt (1) that they all belong to the same life-history, and (2) that they are young *Tetrarhynchids* leading on to the stages shown in figs. 10 and 11.”

Prof. Herdman gives, in support of his theory, a series of figures showing the hypothetical transition from *Tylocephalum ludificans* to *Tetrarhynchus unionifactor* (Report, Part V. p. 21).

Later, a younger *Tetrarhynchus*, 1 mm. in length, was found in the stomach and alimentary canal of the oyster, which Shipley and Hornell (Report V. pp. 87–88) regarded as probably an earlier stage of *Tetrarhynchus unionifactor*, though possibly a distinct form. A single example of a still younger form, which is figured in the Report on Pearl-Production (V., pl. iii. fig. 10), and is described in the text (p. 22) as occurring “encysted in the liver” and in the explanation of the plates as “from cyst between stomach and liver,” appears to go still further towards linking the two forms. This larva is referred to in the text as .53 mm. long. *Tylocephalum ludificans* grows to a much greater size than this without changing its characters; thus the one figured by Shipley and Hornell in the “Parasites of the Pearl-Oyster” (Report, Part II. pl. i. fig. 12), and described as $\times 40$, appears from the size of the figure to be over 1.5 mm. long, and still shows no sign of becoming a *Tetrarhynchus*. And *T. ludificans* quite frequently measures 1 mm. in diameter. This discrepancy in dimensions makes it hard to believe that they are the same organism, and the gap between the *Tylocephalum* form, with its round *Balanoglossus*-like scolex or “myzorhynchus,” and the

Tetrarhynchus form, with its complex proboscides, cannot be said to have been satisfactorily bridged.

Herdman found the *Tetrarhynchus* form much scarcer than the *Tylocephalum* form, and it appears from his Report, Part V. p. 22, that the ratio of the latter to the former is about 200:6. Shipley and Hornell (Report, Part II. p. 79) give the ratio of globular larvæ to undoubted *Tetrarhynchi* as 100:1.

Prof. Herdman's suggested explanation of this, namely, that the globular parasite only occasionally advances to the *Tetrarhynchus* stage, requires, it seems to me, a greater effort of the imagination than the hypothesis that the two worms are distinct forms.

It is hard to conceive of conditions that would lead a small minority of *Tylocephalum ludificans* or *T. minus* to leave their tough fibrous cysts in the peripheral tissues, and migrate to the intestine, there to take on the *Tetrarhynchus* form. It seems to me much easier to regard these as two (or three) distinct species, and their simultaneous presence in one host as a case of parallel infection.

In his latest paper (42, p. 129), Southwell, speaking of these undoubted *Tetrarhynchids*, says:—

“These are by no means rare, and are in almost every case confined to a particular part of the wall of the gut, about one inch from the anus and on the terminal part of the gut. They often occur in clusters of three or four. They are small (about 1 mm.), but appear to be adult in every way, save that strobilization has not commenced. This encysted young *Tetrarhynchid* is quite dissimilar to the globular cysts found in the same oyster. In the latter case, the larvæ are so young that the Cestodian characters are but ill defined. In the former case, a normal and full-grown *Tetrarhynchid* head is present. No stage or stages have been found intermediate between them, and the evidence that they are both stages in the life-history of the same parasite rests on circumstantial evidence and on evidence obtained by feeding experiments.”

And with reference to these feeding experiments, which are referred to below (p. 287), and in which *Tetrarhynchi* were found in Sharks that had been fed on pearl-oysters, Mr. Southwell says (p. 130):—

“The mere fact that the adults were obtained by feeding is in itself almost sufficient to prove that they are the adult of the pearl-inducing worm, for it is difficult to believe that their occurrence in the *Ginglymostoma* was a mere coincidence each year.”

I think there is very good reason to believe that Southwell did, in his feeding experiments, actually transmit *Tetrarhynchus unionifactor* from the Oyster to the Elasmobranch, but it is

difficult to escape the conclusion that the worms found in *Ginglymostoma* were derived from the *Tetrarhynchus* larvæ in or around the alimentary canal of the Oysters, and not from the globular *Tylocephala* in the other tissues, to which Southwell refers when he speaks of the "pearl-inducing worm." To dispute this view, it would be necessary to demonstrate that the *Tetrarhynchus*-stage was not present in the Oysters used.

Shipley remained throughout sceptical about the identity of the supposed pearl-forming larvæ with *Tetrarhynchus unionifactor*. In Part II. of Herdman's Report, p. 86, he says it is most improbable that the young larvæ grow into the *Tetrarhynchus* larva. In their report on the Cestode and Nematode Parasites from the Marine Fishes of Ceylon, Shipley and Hornell say (Pt. V. p. 66):—

"It seems increasingly probable that the pearl-forming Cestode is a *T. unionifactor*, but this has not yet been proved."

Shipley and Hornell, in Herdman's Report, Part V. p. 98, offer the following hypothetical life-history:—

"Of the given number of larvæ which enter at a very early stage into the body of the Oyster a certain number arrive in the mantle and other tissues, acquire an ectodermal sac and there encyst, and find a costly grave in the developing pearl." [The ectodermal sac around these parasites is so far purely hypothetical and has never been demonstrated.—H. L. J.] "The remainder, however, reach the alimentary canal and grow and flourish there. When they attain the dimensions of the stages described in Part II. they leave the alimentary canal and encyst, usually upon the outer surface of the intestine. Now they are too big for enclosure in a pearl, and they can wait without anxiety for the advent of their second host (*Rhinoptera jaranica*), within whose intestine they rapidly become sexually mature."

It would seem to the present writer much simpler to set aside, for a while, the hypothesis that *Tylocephalum ludificans* and *T. minus* are younger stages of a *Tetrarhynchus*, and to seek for their adult stages among the members of the genus *Tylocephalum*, or allied types described as new genera, occurring in oyster-eating Elasmobranchs. Shipley and Hornell have already described a number of these, which I give below:—

*†*Tylocephalum* (*Tetragonocephalum*) *trygonis* (Report, Part III. p. 51 and Part V. pp. 48 & 83). *Habitat*: intestine of *Trygon walga* and *Aëtobatis narinari*. Diameter of head 0.03 mm.

**Tylocephalum* (*Tetragonocephalum*) *aëtobatidis* (Report, Part III. p. 52 and Part V. p. 48). Intestine of *Aëtobatis narinari*. Diameter of head 1.5 mm.

†*Tylocephalum dierama* (Report V. p. 59). Intestine of *Myliobatis maculata*. Diameter of head .6 mm.

Tylocephalum kuhli (Report V. p. 72). Intestine of *Trygon kuhli*. Head apparently about .5 mm. in diameter.

†*Tylocephalum uarnak* (Report V. p. 76). Intestine of *Trygon uarnak* and *Trygon walga*. Head apparently about 1 mm. in diameter.

*†*Cephalobothrium aëtobatidis* (Report V. p. 44). Spiral valve of *Aëtobatis narinari*. Diameter of head .5 mm.

*†*Kystocephalus translucens* (Report V. p. 46). Intestine of *Aëtobatis narinari*. Diameter of head .4 mm.

**Eniochobothrium gracile* (Report V. p. 64). Intestine of *Rhinoptera javanica*. Head appears to measure about .4 mm. in diameter.

*†*Tiarabothrium javanicum* (Report V. p. 67). Intestine of *Rhinoptera javanica*. Head 1 mm. broad.

To these may be added two forms described by Mr. Southwell (41 a), viz.:—

Cephalobothrium abruptum, from the spiral valve of *Pteroplatea micrura*. Head 1.2 mm. broad.

Cephalobothrium variabile, from the intestine of *Pristes cuspidatus*. Head 1 mm. broad.

The forms marked * occur in hosts which are known to feed on pearl-oysters. Those marked † I have been able to examine through the courtesy of Dr. Shipley. It is impossible not to be struck with the resemblance between the heads of some of the above species and the parasites which Herdman associates with pearl-formation. Compare, for example, the head of *Cephalobothrium aëtobatidis*, figured in Part V. (Cestoda) plate i. figs. 1-4, with some of the figures of *Tylocephalum ludificans* in the chapter on Pearl-Production (Part V. Pearl-Production, plate iii.) or the heads of *Tylocephala* (*Tetragonocephala*) as figured on the plate of Part III. (Parasites), and in the article on Cestodes in Part V. (pl. v. figs. 76-7), with some of the figures in the article on the parasites of the Pearl-Oyster in Part II. Or, again, compare the section of *Tylocephalum ludificans* from the pearl-oyster shown on Pl. XLVI. fig. 58, with the head of *T. uarnak*, Pl. XLVII. fig. 65, drawn from one of Dr. Shipley's slides, as a representative of the genus *Tylocephalum*, taking into account the difference that in the former the myzorhynchus is withdrawn within the collar, whereas in the latter it is fully protruded. Or compare Pl. XLVI. fig. 59, a section of *T. ludificans* from the pearl-oyster, with the head of *Cephalobothrium aëtobatidis*, from one of Dr. Shipley's slides shown on Pl. XLVII. fig. 66. Similarly, compare figs. 58 & 59 with the worm shown in figs. 61 & 62 on Pl. XLVII., also from *Aëtobatis narinari* (the final host of Seurat's larva) ‡. I think the worm shown in figs. 60-64 is quite probably

‡ It is strange that the oyster-eating habits of this Ray do not seem to have been recorded in these Ceylon researches.

the adult of *Tylocephalum ludificans*—in fact, the only difference appears to be the presence of the four lateral suckers, which are absent in the larva, but which may well not develop till its transference to the final host.

Comparison of these figures makes one feel doubtful whether the generic distinction between *Tylocephalum* and *Cephalobothrium* is a valid one, or whether the conditions shown in figs. 58 & 65, and in figs. 59 & 66, are not merely the expression of a uniform type of myzorhynchus in different stages of contraction, as suggested diagrammatically in the following text-figure.

Text fig. 34.

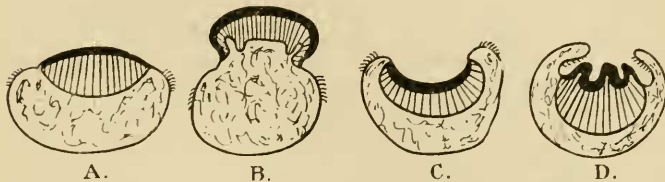


Diagram illustrating the relationship between the condition of the myzorhynchus in *Tylocephalum* and *Cephalobothrium*. A, a generalised scheme of a scolex such as the *Tylocephala* of the pearl-oyster. B, by protrusion of the myzorhynchus, the outer surface of the same being tense, the inner surface thrown into folds, the *Tylocephalum*-form may be produced (compare Pl. XLVII, fig. 65). C, the partial retraction of the myzorhynchus to form a sucker-like disk gives the *Cephalobothrium* condition (compare Pl. XLVI, fig. 59 and Pl. XLVII, fig. 66). D, the myzorhynchus retracted within its collar, with its outer surface thrown into folds, as is characteristic of many of the larvae of *Tylocephalum ludificans* found in the pearl-oyster, and of the adult worm figured on Pl. XLVII, figs. 61-62.

It does not necessarily follow that any of the above mentioned worms actually represents the final stage of *Tylocephalum ludificans* or *T. minus*, though I think there are considerable grounds for regarding the worm shown at figs. 60-64 as the former; but it certainly appears more probable that these final stages will be found among this class of parasites rather than among the *Tetrarhynchi*, and it is strange that the position that the *Tetrarhynchus unionifactor* hypothesis may be wrong has never been seriously faced and a fresh start made on the above lines.

It is not known how *Tylocephalum ludificans* and *Tylocephalum minus* enter the Pearl-Oyster; but Hornell found, in tow-netting on the Muttuvarattu Paar on the 19th November, 1902, a free-swimming larva, .37 mm. long when extended, which is figured in Prof. Herdman's Report (Part II. Parasites of the Pearl-Oyster, plate i. fig. 1 a-h). This larva certainly suggests an earlier stage of *Tylocephalum ludificans*, and one of the chief difficulties in the way of accepting it disappears if this worm is dissociated from the genus *Tetrarhynchus*, the normal habit of which is to enter the digestive canal with the food while still in the egg-

stage. (The fact that the undoubted *Tetrarhynchi* in the pearl-oyster occur in and around the digestive canal suggests that they follow the normal course and are swallowed in the egg-stage, and first hatch out in the intestine of the oyster.) Mr. Southwell states (Ceylon Marine Biological Reports, Part IV. No. 6, p. 169, 1910) that this free-swimming larva has not been seen since it was first discovered (see also 42, p. 127).

I may here mention that one of Prof. Herdman's slides which I examined shows an interesting phase in the biology of these supposed pearl-inducing Cestodes which may have escaped him. It shows a small Cestode, 0.12 mm. long, with myzorhynchus and collar fully developed, clearly in the act of passing through the tissues. This may possibly represent the young of either form, when first entering the oyster, or it may be a case of voluntary or accidental migration by *Tylocephalum minus* (Pl. XXXIII. fig. 1). Dr. Willey (48, p. 50) records a similarly free larva seen moving about in the liver of a species of *Venus*.

To return to the life-history of the true *Tetrarhynchus unionifactor*, Shipley and Hornell have shown without doubt that the adult sexually mature worm occurs in the Ray, *Rhinoptera javanica* (Herdman's Report V. Cestodes, pp. 65-66). The identification of the final host as *Rhinoptera javanica* is announced by Mr. Hornell in a postscript to his Report on the November Inspection of the Pearl Banks, 1904 (20, p. 8). Mr. Southwell (42, p. 130) gives *Taniura melanospilos* as another host.

Professor Herdman in his Royal Institution Lecture, and in Pt. I. p. 12 of his Report, claimed the File-fish, *Balistes*, as an intermediate host; but Shipley and Hornell, in Herdman's Report, Part II. p. 83, say that "a more minute examination, however, renders the connection between the parasites of the pearl-oyster and those of the file-fish a doubtful one"; and the immature *Tetrarhynchi* found in this fish are described as distinct species under the names of *Tetrarhynchus balistidis* and *T. pinnae*. Prof. Herdman sums up the position in the article on Pearl-Production (Report V. p. 24) by saying:—

"No fresh light has been thrown upon the possible occurrence of an immature stage in *Balistes* (which is eaten by the large rays), and although that intermediate host may not be necessary to the life-history, since the rays also feed upon pearl-oysters, still there is nothing in the observed facts to forbid the existence of such a stage, and it is not unusual in *Tetrarhynchids* to have two fish-hosts, an intermediate Teleostean which is devoured by a final Elasmobranch."

Mr. Southwell's subsequent investigations confirm Prof. Herdman's view that *Balistes* occurs as a collateral intermediate host or "carrier"; he says (42, p. 132):—"It is certain that my encysted *Tetrarhynchus unionifactor* from *Balistes* is not the same species as those described" (*i. e.* by Shipley and Hornell

from *Balistes*); and he claims that they are "exactly similar to the encysted Tetrarhynchid found in the oyster"—i. e. the true *Tetrarhynchus unionifactor*, except that they are slightly larger. Mr. Southwell is satisfied that they are derived from the oysters eaten by *Balistes*, and thinks that they are derived from both the genuine Tetrarhynchids in the Oyster's intestine and from the globular cysts in its tissues. He considers that if *Balistes* is eaten by an Elasmobranch, the young worms become adult; but *Balistes* is not a *necessary* host, it is merely a "carrier."

Johnstone (26 b) confirms the view that the Teleostean host is a collateral one, and not a normal stage in the life-history, by his recent researches on the European *Tetrarhynchus erinaceus*. The adult stage of this worm occurs in various species of Skates and Rays, and the first host is probably some Invertebrate. Johnstone regards the frequent occurrence of a larval form of this worm in Teleosts as a "cul-de-sac" stage, due to the first host being eaten by the wrong fish; and as normally leading no farther, but ending in degeneration.

Mr. Southwell has further shown (38) that the adult *Tetrarhynchus unionifactor* occurs also in the Shark, *Ginglymostoma concolor*. This fish was doctored with Male-fern and castor-oil, and subsequently fed on pearl-oysters; but Mr. Southwell does not claim that the *Tetrarhynchi* were actually derived from these pearl-oysters, though he is inclined to think they were. The same experiment was subsequently repeated [Southwell (41)] and *Tetrarhynchus unionifactor* was again found in *Ginglymostoma concolor*; and while Mr. Southwell admits that his results are not altogether conclusive, it seems highly probable that the infection was in fact induced by his feeding experiment. There is nothing, however, to show that the adult tapeworms in *Ginglymostoma* were derived from the globular cysts in the oysters; it seems more probable that they were derived from the *Tetrarhynchi* in the oysters' intestines.

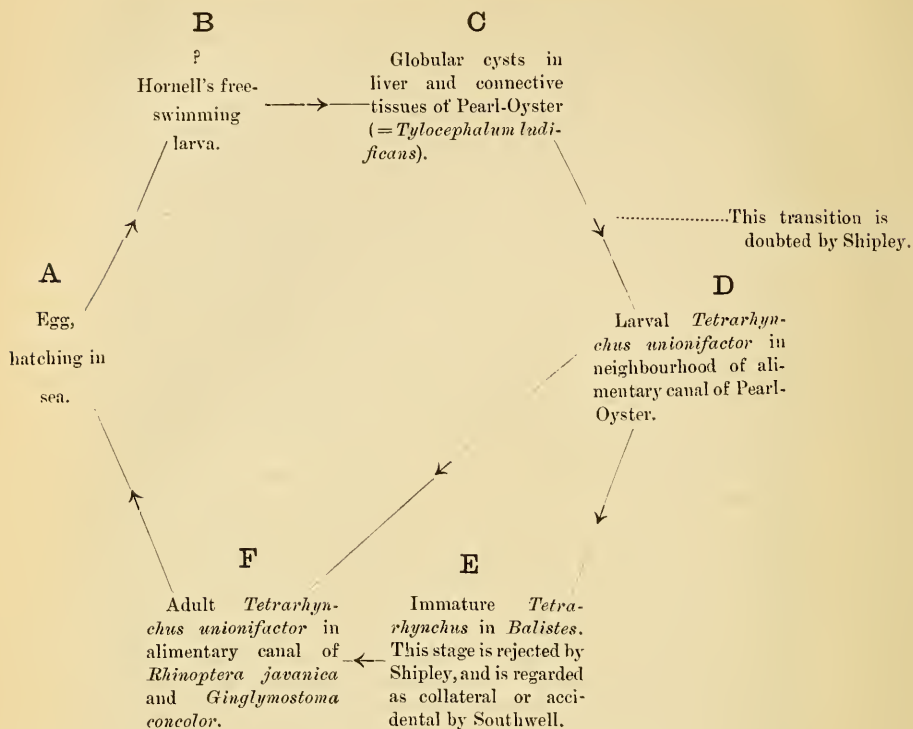
These elaborate experiments are, of course, chiefly of academic interest, in the absence of proof (1) that the *Tetrarhynchi* are a later stage of the globular cysts, and (2) that the latter are concerned in pearl-formation.

To sum up, then, the gap between (a) the resting scolex enclosed in its tough fibrous cyst in the connective tissues of the Pearl-Oyster and strongly suggesting by analogy with other forms—notably Senrat's larva—a young *Tylocephalum*, awaiting and ready for its final host to devour the tissues which contain it, and (b) the equally expectant, but much larger, *Tetrarhynchus unionifactor* in the wall of the oyster's intestine, has not yet been bridged.

I set out below diagrammatically the conclusions of Herdman, Hornell, Shipley, and Southwell, as to the probable life-history of these parasites, and also, for purposes of comparison, my own attempt at an interpretation of the facts.

DIAGRAM illustrating Prof. Herdman's conclusions as to the
life-history of the parasites.

Species I. *Tetrarhynchus unionifactor*.



Species II. "*Tetrarhynchus* sp."

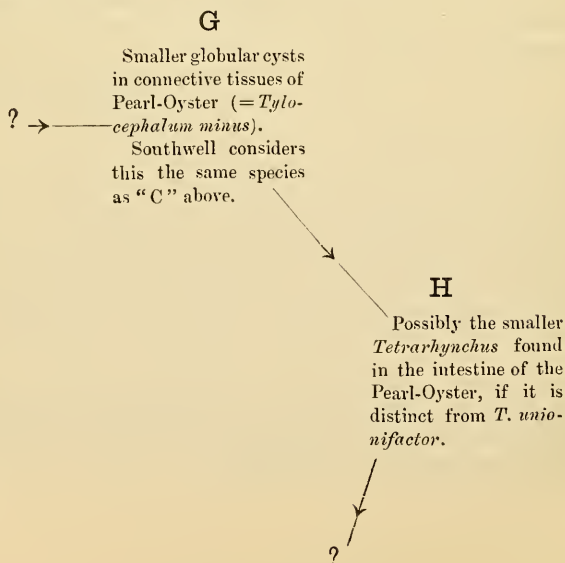
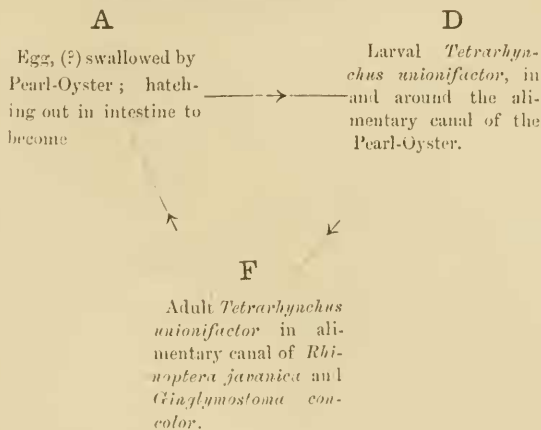


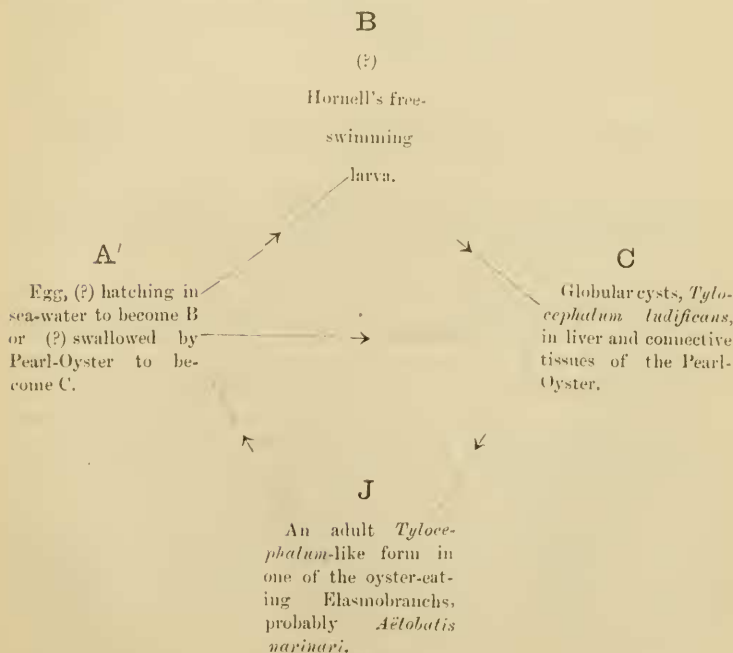
DIAGRAM illustrating my hypothesis of the relations of the several forms and stages of Cestode larvæ described in Herdman's Report.

(The letters correspond to those in the above diagrams. Those marked A', A'', B', and J' are regarded as parallel stages to A, B, and J.)

Species I. *Tetrarhynchus unionifactor*.



Species II. *Tylocephalum ludificans*.

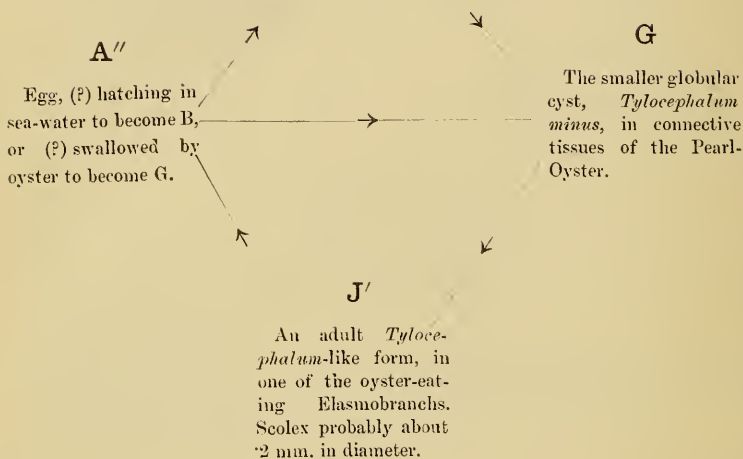


Species III. *Tylocephalum minus*.

B'

(?)

A free-swimming larva,
like B above, only
smaller.



(5) DESCRIPTION OF THE TWO GLOBULAR CESTODE LARVÆ FROM THE CEYLON PEARL-OYSTER.

I append the following descriptions. They are certainly incomplete, being based on examination of preserved material and on the descriptions of previous writers:—

***Tylocephalum ludificans*, sp. n.** (The larger globular larva; the supposed pearl-producing worm.) (Pl. XLVI. figs. 58 (type) & 59.)

The type, fig. 58, is in Slide 94 in Prof. Herdman's Collection.

Tetrarhynchus unionifactor (young) Herdman. (For other figures see Herdman's Report, Part II. Parasites of the Pearl-Oyster, pl. i. figs. 12 & 13; Part V. Pearl-Production, pl. ii. figs. 11, 12, & 17b, pl. iii. figs. 1-5, 6, 7, 8, & 9; also Part V. p. 21.)

Globular Cestode larvæ, with rostrum or *myzorhynchus* (Linton) retractile within a denticulated collar. Form more elongated when liberated from capsule; length 0.5 to 1.5 mm. Average diameter of seven specimens sectioned on Prof. Herdman's slides and examined by the writer, 0.78 mm.

Myzorhynchus uniformly muscular, without obvious division into muscular tracts; retractile within an annular collar; in section it may appear either conical, lenticular, or flattened, concave and sucker-like; protrudes as a conical papilla when in locomotion. This anterior muscular region, including the collar, is about one-third of the total length of the larva when extended. The whole myzorhynchus can be protruded, the collar then forming an annulus around it.

Collar or cephalic sheath muscular with denticulated cuticle, the denticles tricuspid. (Herdman's Report, Part II. (Shipley & Hornell, Parasites of the Pearl-Oyster) pl. i. figs. 10, 11, & 14, pl. ii. fig. 18; also Pt. V. (Pearl-Production) pl. iii. fig. 9.)

The denticles measure from 3μ to 5μ in diameter.

Hinder part of the larva centrally parenchymatous, the parenchyma containing the calcareous corpuscles characteristic of Cestode larvæ, peripherally more muscular.

The hinder part of the body is covered by a thick, radially marked epicuticle, permeated by numerous closely-set tubuli, and suggesting on superficial examination a coat of cilia. This epicuticle varies in thickness but is generally about 0.03 mm. thick, and the true cuticle lies under it.

This form is distinguished from the next described worm by its larger size (Herdman gives the size as about six times that of the smaller form), the undivided musculature of the myzorhynchus, and the wider and more open character of the collar-sheath of the myzorhynchus in the resting-stage.

Habit. Resting in spherical fibrous cysts, derived from the connective tissue of the host, in the Ceylon Pearl-Oyster, *Margaritifera vulgaris*. Most frequent in the visceral mass, notably the liver.

Habitat. Gulf of Manaar (Herdman & Hornell). Trincomalee (Willey).

The following is a description of a worm which I regard as in all probability the adult of this larva. The single specimen was obtained from the spiral intestine of *Ætobatis narinari*, by Mr. Hornell, on 4th January 1905, and had apparently been overlooked by Mr. Shipley among some duplicate specimens of *Kystocephalus translucens*, along with which I found it when examining Dr. Shipley's material. After it had been cleared and examined as a transparent object, Dr. Shipley very kindly allowed me to have sections cut from it to compare with those of the larva in the pearl-oyster.

(?) Adult of *Tylocephalus ludificans*, sp. n. (Pls. XLVI. & XLVII. figs. 60-64.) Length 12 mm. Head 0.6 mm. long by 0.5 mm. broad; pyriform, slightly broader in front than behind; transition from head to neck not very sharply defined. The myzorhynchus in this specimen is retracted within its sheath, as is usually the case with the larva in the pearl-oyster; it is about .3 mm. in diameter. Around the head are four marginal suckers about .125 mm. in diameter. Proglottides about 140 in number, increasing but little in breadth from before backwards; they

begin to increase notably in length from about the 85th backwards. The largest hindmost segments are about .5 mm. long, and slightly longer than broad (fig. 64). The armature of the collar *a* (in figs. 61-63) is similar to that of the larva. In section (fig. 62) the myzorhynchus is seen to be retracted in such a way that its anterior surface is thrown into folds, thus resembling the condition of the larva shown in fig. 59. The only point in which the head of this worm appears to differ from the larva in the pearl-oyster is in the presence of the four marginal suckers, which may well be a feature first acquired in the final host.

Tylocephalum minus, sp. n. (Plate XXXIII. fig. 2.) (The smaller globular larva, which Prof. Herdman thinks may also be concerned in pearl-formation.)

(*Tetrarhynchus* sp., Herdman.)

For other figures see Herdman's Report (Pearl Production), Part V. pl. ii. figs. 1-3, 17A, 18-22; also text-figure 3, p. 19.

Diameter of resting parasite in cyst from 0.07 to 0.2 mm. Average diameter of 40 examples shown on Prof. Herdman's slides and measured by the present writer, 0.14 mm. Body sub-globular, consisting, as in *T. ludificans*, of an anterior muscular and a posterior parenchymatous part, the anterior muscular portion (myzorhynchus) consisting of a conical papilla in a cup- or flask-shaped depression formed by the surrounding muscular collar or sheath. As a rule, in preserved specimens, the opening of this depression seems relatively narrower, and the papilla more conical and less flattened than in the previous species. The musculature of the myzorhynchus shows, in some examples, a tendency to break up into four longitudinal tracts. In young examples the myzorhynchus may be barely differentiated. Cuticular spines are present on the collar, but they are smaller and relatively finer than in *T. ludificans*. The epicuticle is about .01 mm. thick.

This form is distinguished from *T. ludificans* by its smaller size and finer armature of the collar, and by the tendency of the myzorhynchus musculature to break up into four strands. It is regarded by Southwell as the same species as *T. ludificans*.

Habit. Resting in spherical fibrous cysts in the connective tissues of *Margaritifera vulgaris*, occurring in the visceral mass, mantle, gills, etc.

Habitat. Gulf of Manaar.

Prof. Herdman, while he regards the form here named *T. ludificans* as the pearl-producer *par excellence*, considers that the present species too "may occasionally form the nuclei of pearls" (Report V. p. 22).

Particulars of the structure of both these forms are given on pp. 79-82 of Part II. of Prof. Herdman's Report.

(6) OBSERVATIONS ON THE STRUCTURE OF THE FIBROUS CYSTS SURROUNDING THE CESTODE LARVÆ IN *MARIGARTIFERA VULGARIS*.

I may here add a few notes on the structure of the investing cysts of *Tylocephalum ludificans* and *T. minus*, based upon my examination of Professor Herdman's slides and Dr. Kelaart's material.

In the earliest stages the cyst may be scarcely differentiated from the surrounding tissue, and about 0.01 or 0.02 mm. thick. This condition I find specially associated with a little-developed phase of the smaller larva *T. minus*, measuring 0.08 mm. in diameter, and resembling B in figure 4 on p. 21 of Part V. of Herdman's Report. In such thin cysts the nuclei of the fibres are distinct, though hardly more so than those of the general connective tissue of the oyster. One or two examples at this and later stages were found in the muscular tissue without any surrounding cysts at all, beyond a little of the interstitial tissue of the muscle-bundles (Pl. XXXIII. fig. 2). As the fibrous capsule becomes thicker the nuclei appear at first to become more abundant, and this may well be associated with the growth and multiplication of the fibres. As a rule, the thicker cysts (0.2 mm. thick and over) seem to be less densely nucleated, and may even show very few nuclei, especially when they become highly areolar and œdematous. The outer part of the cyst is usually ordinary areolar connective tissue, with branched and anastomosing fibres passing over, often quite imperceptibly, into the general connective tissue of the body, such as occurs between the tubules of the liver. The cyst is, however, typically lined with several layers of more regular parallel fibres, with abundant nuclei. In some cases the fibres seem to coalesce to form a dense almost gristly substance, without obvious nuclei.

The nuclei of the fibres are long and narrow, and are situated on their outside edges.

In some cases the cyst appears to be entirely without nuclei, and in such cases the fibres are often very thick, measuring as much as 10 or 15 μ in diameter.

There is never, so far as I can see, any trace of a lining epithelium, though the cyst may be lined with a layer of granules, possibly derived from the Cestode or from the leucocytes of the blood.

In certain cases large dark-staining bodies were seen in the areolæ of the fibrous connective tissue of the cyst, which suggested parasitic Sporozoa, but the preparations did not allow of detailed examination.

A typical section of the cyst of the smaller parasite, *Tylocephalum minus*, is shown on Pl. XXXIII. fig. 3.

This condition of things is very different to that which is found in the case of the pearl-inducing Trematode of *Mytilus* described in my paper (25). Here the parasite is surrounded by an

epidermal sac (*l. c.* pl. xv. fig. 5) of the same nature as the outer shell-secreting epidermis. In such a case it is easy to understand how the parasite, when it dies, becomes encased in a pearl, laid down, layer upon layer, by this epithelium*.

(7) MATERIALS AVAILABLE FOR THE PRESENT INVESTIGATIONS.

I have throughout these investigations been seriously handicapped by the extreme difficulty of obtaining material. Many of the points which remain obscure could probably be cleared up if I could obtain properly preserved specimens of pearl-bearing oysters from the Gulf of Manaar. Unfortunately, I have been quite unable to obtain these.

I endeavoured to do so through the Ceylon Company of Pearl Fishers, Ltd., and Mr. Southwell, but without success, Mr. Southwell replying that there were no oysters on the banks and that his own preserved material was finished. The Company, however, kindly forwarded to me a suggestion made by Mr. Southwell to the following effect:—

“As it is probable Dr. Jameson requires Ceylon pearls (with the particular parasite giving rise to same), I would suggest that in order to ensure that the pearls are from Ceylon that they be bought here. I shall be glad to purchase pearls for Dr. Jameson, if he will give me some idea what to get and how much to spend.”

I gladly availed myself of this offer, and asked Mr. Southwell to spend five pounds in the purchase of “cyst-pearls.” For this sum he procured from a local jeweller a parcel of 21 small “fine” pearls, which I received in February 1911.

* I must here incidentally refer to a quite erroneous interpretation which was placed upon the expression of my views as to the origin of the sac in *Mytilus* in my paper above referred to.

My account of the development of this sac on p. 149 appears to have been taken by Herdman and by Boutan (3 & 4) to imply that I thought the sac arose from the mesoblastic connective-tissue elements of the mantle. As I explained in a letter to Prof. Herdman, which he was good enough to publish, as showing my views, on p. 9 of Part V. of his Report, I never had any doubt that the sac was a true epidermis. What I wished in my paper to emphasize was that in *Mytilus* it appeared to arise independently of, and not in continuity with, the outer epidermal epithelium, perhaps from in-wandering epidermal cells, perhaps from more deeply seated elements of epiblastic origin, some of which (*e. g.* certain flask-shaped glands in *Margaritifera*, see Pl. XLI. fig. 33) appear to project below the basement-membrane. Had I dreamed that I should have been suspected of attempting to promulgate heretical views on the doctrine of the immutability of the three primary germinal layers, I would have been more cautious in the choice of my phrases. But even if my wording in that paper was unintentionally somewhat ambiguous, my *résumé* of my work in ‘Nature’ (26) should have cleared away any misconception, for in that paper I definitely stated (p. 280) that “a true pearl is laid down in a closed sac of the shell-secreting epithelium, embedded in the subepidermal tissue of the mantle and completely cut off from the outer epithelium itself. . . . Such a sac, with its contained pearl, may be compared to a human atheroma cyst.” I have not yet reached the stage at which I can add to what I said in 1902 about the actual mode of origin of the epidermal sac in *Mytilus*, but I hope before long to be able to contribute some more facts on the subject.

I then tried the Colombo Museum in the hope of getting some oysters with pearls *in situ*, but Dr. Pearson had no preserved material to spare. I tried to obtain material from Madras from Mr. Hornell, but he wrote me, in January 1911, that his own material was exhausted, and that he would not be able to obtain any more till the next inspection, a year later. However, H.H. the Jam Saheb of Nawanagar most kindly sent me some preserved specimens of this species with pearls *in situ* from the Gulf of Kutch, and I hope, in a later publication, to be able to put forward some observations on the actual process of pearl-production, based on these.

The following material was available for these investigations :—

(i.) Twenty-one pearls bought in Ceylon. It is, of course, possible, though not probable, that some of these originally came from elsewhere, *e. g.* the Persian Gulf *via* Bombay, but they, or at least the great majority of them, were certainly derived from *M. vulgaris*, the pearls of which have a characteristic colour and lustre quite different from that of the pearls found in *M. margaritifera* and *M. maxima*.

They were small "fine pearls," mostly spherical, a few oval or slightly lenticular. One was a brown pearl formed in the mantle-margin from the prismatic substance. They were all decalcified and examined whole, cleared in oil of cloves, and drawn. They were then sectioned (except in the case of three examples which were preserved whole). Their nuclei were in no cases Cestodes; they usually contained a cavity with a few granules surrounded by spherocrystal-like matter, allied to or identical with the "repair-substances" described below. In several cases, however, the actual nucleus was a grain of sand. These specimens are preserved as preparations XL, XLII, XLIII, XLIV, XLV, XLVII, LI, LII, LIII, LIV, LIV A, LIV B, LIV C, LIV D, LIV E, LIV F, LIV G, LIV H, LIV I, LIV J, and LIV K (Pls. XLI.-XLIII. figs. 35-45 and Pls. XLV., XLVI. figs. 50-57).

(ii.) *Dr. Kelaart's Material in the British Museum.*

In 1901, when I was investigating the origin of pearls in *Mytilus*, Mr. E. A. Smith, I.S.O., allowed me to examine five old specimens of the Ceylon Pearl-Oyster from Dr. Kelaart's collections in the British Museum. Mr. Smith very kindly allowed me to make further use of some of this material for the present investigations.

The specimens are labelled :—

- "1 specimen of pearls in ovaria,
- 3 specimens of pearls in mantle,
- 1 specimen of ova of Entozoa in liver of *Meleagrina margaritifera*."

The specimen with "pearls in ovaria" was a pearl-oyster with

a quantity of clustered pearls, mostly of the baroque and seed-pearl classes, in the visceral mass. The three examples with "pearls in mantle" showed pearls, chiefly in the regions of the levator muscles, though some were in the non-muscular parts of the visceral body-wall. The specimen with "ova of Entozoa in liver" is interesting, as showing the Cestode, *Tylocephalum ludificans*, in its whitish fibrous pearl-like cysts, which on superficial examination suggest "eggs." It was in this specimen, in 1901, prior to Prof. Herdman's departure for Ceylon, that I first became acquainted with these larvæ, which Prof. Herdman identified as the cause of cyst-pearls. The few observations that I was able to make at that time led me to the conclusion that there was no evidence that this parasite was concerned in pearl-formation*, a conclusion that I have, so far, seen no sufficient cause for modifying.

Owing to their age, the state of preservation of these specimens was naturally somewhat defective, and for real detailed histological work upon the pearl-producing tissues they were quite useless.

Many of the pearls in these specimens had fallen out of their sacs and lay in the bottom of the jar. Some of these, along with others picked out of the tissue, numbering 22 in all, were decalcified and examined in oil of cloves, and six of them were then sectioned and further examined. [Preparations XIV, XV, XVI, LXXI c, LXXI g, LXXI j (Pls. XXXVII., XXXVIII. figs. 16-18 and Pl. XLIV. figs. 46-48).]

A large piece of tissue in the wall of the visceral mass, measuring about $5 \times 5 \times 4$ mm., and containing no less than 16 pearls, was cut out from the specimen with "pearls in ovary," decalcified, stained with borax carmine and indigo carmine, and sectioned [Preparation XXVIII (Pl. XXXVII. figs. 14, 15)]. These pearls were all of the class which I refer to provisionally (see below) as muscle-pearls, and were mostly formed around central cavities.

(iii.) *Unlabelled Material in the British Museum.*

Mr. Smith also allowed me to examine two unlabelled specimens of *Margaritifera vulgaris* in the British Museum, the history of which is unknown. One of them was with the example in spirit now on show in the Museum, and was accompanied by its shell. It is from this example that preparations XXVII and XXIX were cut. It contained a large number of muscle-pearls and what Prof. Herdman calls "calcospherules" in the left mantle-lobe, and in the region of the adductor muscle on the left side. The other example had been removed from its shell. It contained a large number of clustered pearls, of all sizes, in the right mantle-lobe. The tubes in which the specimens were preserved contained also a lot of loose pearls which had dropped out of both these specimens.

* In 1902 (25), p. 149, I pointed out that Cestode larvæ were not surrounded by a pearl-sac.

From the first of these specimens the following preparations were made:—

(a) Preparation XXVII, a piece of tissue cut from the borderland between the mantle and the adductor in the first of the above specimens. This piece measured about $7 \times 5 \times 4$ mm., and contained 36 small pearls and numerous so-called "calcospherules." The preservation was so bad that no differential staining of the soft tissues could be obtained, all parts reacted alike to the stains used. [Preparation XXVII (Pls. XXXVIII., XXXIX. figs. 19–21, Pl. XL. figs. 24–27.)]

(b) Preparation XXIX was a piece of the mantle of the same individual near the margin, containing 17 so-called "calcospherules," one of which is becoming coated over with nacre (Pl. XXXIX. figs. 22 & 23). The figures were made from the whole object—fig. 22 representing it as it was before decalcification, fig. 23 after it had been decalcified; both as seen when cleared with oil of cloves. The preparation was then sectioned, but the state of preservation did not allow of the relations of the "calcospherules" to the tissues being investigated in this instance. A piece of tissue was also cut from the second of these specimens, decalcified, examined entire, and sectioned. It contained about 20 small pearls and numerous so-called "calcospherules." [Preparation XXIV.]

Forty pearls, of varying sizes, some lying loose in the bottoms of the jars containing the specimens, others taken from the tissues, were decalcified and examined in oil of cloves. Of these, six were sectioned and further examined. [Preparations XXIII, LXIII (A, B, & L), and LXVI (A & I) (Pls. XXXIX.–XLI. & XLIV. figs. 21 a, 28, 31, 32, 49).]

All the pearls from these specimens I refer to the class called by Herdman "Muscle-Pearls."

(iv.) *Three Specimens of the Pearl-Oyster collected by Professor Herdman in 1902.*

Prof. Herdman, at the request of the Ceylon Company of Pearl Fishers, Ltd., very kindly allowed me to examine his material (see (v.) below) and handed me three specimens of the Pearl-Oyster, each of which contained a small "muscle-pearl" at the point of insertion of one of the levators of the foot. The pieces of tissue containing these three pearls were cut out, decalcified, and sectioned [Preparations IV, VI, and VII (Pl. XXXV. fig. 8)]. Although these specimens had, apparently, been preserved in formalin, which is not the most satisfactory preservative for histological purposes, they showed quite a lot of histological detail, and enabled me to form some idea of the mode of origin of muscle-pearls, and of the curious cyst-like bodies which precede them.

(v.) *Professor Herdman's Slides.*

As stated above, Prof. Herdman very kindly allowed me to examine his slides, which he sent to me a few at a time.

Most of these slides were preparations showing the parasitic Cestodes in the tissues of the oyster, but there were also a number of preparations of pearls, sectioned *in situ* in the tissues, showing in all about 25 pearls, 21 of which showed their nuclei more or less distinctly. The nuclei of these pearls were of very different characters, but in no case could I identify a Cestode larva in the centre of a pearl.

(vi.) *Three Specimens from the Persian Gulf.*

In September 1903, Mr. J. Calcott Gaskin, Assistant Political Agent at Bahrein, Persian Gulf, sent me 32 specimens of *Margaritifera vulgaris* from Bahrein preserved in alcohol. Out of 20 of these that I opened, 3 contained pearls, which, from their position, could obviously be classified as "cyst-pearls" (Herdman). Two contained a single pearl each, that in the first being about 2.5 mm. in diameter, situate in the left mantle-lobe, above the anterior end of the attachment of the gills [Preparation LXIV A], that in the second [Preparation LXIV B] being about 1 mm. in diameter and situate in the body-wall over the stomach. The third specimen had two small pearls, about 2 mm. apart, in the wall of the visceral mass, away from all muscle-impressions [LXIV C]. All these pearls were decalcified *in situ* in the tissues and sectioned. Their centres are described below.

In addition to the above the following pearls were decalcified and examined. More detailed particulars are given under the descriptions of the centres of individual pearls, given below:—

(vii.) Dry unlabelled pearls, probably from Ceylon, in the British Museum, three examples were decalcified.

(viii.) Mixed lot of pearls; given to me by Mr. Max Mayer, mostly from *Margaritifera vulgaris*; 115 were decalcified, of these 8 were sectioned.

(ix.) A collection of pearls from the last Ceylon pearl fishery, given to me by Mr. E. Hopkins. Fifteen were decalcified, and four of these were sectioned.

(x.) A collection of pearl-oysters, with pearls *in situ*, from the Gulf of Kutch; these are referred to above. Up to the time of writing, 18 pearls from these specimens have been decalcified, and 13 of them sectioned.

(xi.) Two pearls from *Margaritifera vulgaris*, from the Mediterranean, given to me by Professor Raphael Dubois.

(xii.) Five pearls from *Margaritifera vulgaris*, from New Caledonia, given to me by Professor L. G. Seurat.

(xiii.) A pearl from *Margaritifera vulgaris*, from Madagascar, given to me by Professor Seurat.

(xiv.) Two pearls from *Margaritifera vulgaris*, from Papua, from the Imperial Institute.

(xv.) Twenty pearls from *Placuna placenta*, from Lake Tampalakamam, Ceylon, from the Imperial Institute.

(xvi.) About a dozen pearls from *Margaritifera margaritifera* var. *cumingii*, from the Gambier Archipelago.

In the course of these observations, apart from studies on the structure and formation of pearls in other forms, 356 pearls derived, with perhaps a few exceptions, from *Margaritifera vulgaris*, chiefly from Ceylon, have been decalcified and examined, 175 of these having been studied in sections.

(8) METHODS.

For decalcification, whether the pearls were free or *in situ*, preserved in alcohol or dry, I found alcohol of about 50 per cent. strength, to which a few drops of nitric acid had been added, was the best. Of course, a preserved pearl never decalcifies as well as a fresh one. In the case of dry pearls, where only the nucleus and central parts are required for examination, it is often an advantage, during decalcification, to strip off the outer layers of conchyolin, thus facilitating the penetration of the reagent. Bubbles of carbon dioxide, generated in the process of decalcification between the conchyolin-layers, cause a great deal of trouble, especially in old dry pearls, where the conchyolin seems to be particularly leathery and impermeable. Such bubbles often greatly distort the normal structure of the pearl, as seen in section, by tearing the conchyolin-layers apart and causing great spaces between them. The most suitable reagent for expelling the bubbles is absolute alcohol, in which the decalcified pearl is placed for a few days. In some cases, however, it was necessary to extract the gas under an air-pump.

For staining decalcified pearls hæmatoxylin was used, also borax carmine. Sections of the pearls in the tissues were stained sometimes with borax carmine and picro-indigo-carmine*, sometimes with hæmatoxylin and eosin or orange, occasionally with other reagents.

(9) STRUCTURE OF THE SHELL-SUBSTANCES.

The shell of *Margaritifera* consists of the following parts:—

- (i.) the outermost layer or so-called Periostracum;
- (ii.) the prismatic layer, forming with (1) the “back” of the shell, the fragile, dark-coloured “lip,” and the lappet-like processes of the margin;

* Borax carmine (Grenacher's) in bulk:—

Picro-indigo-carmine as under, on the slide.

A. Saturated solution of picric acid in 90 per cent. alcohol.

B. Saturated solution of indigo-carmine (Grubler) in 70 per cent. alcohol.

1 part of A, 2 parts of B, 6 parts of 70 per cent. alcohol.

- (iii.) the Nacre or Mother-of-Pearl, forming the lining and the bulk of the shell ;
- (iv.) the Hypostracum, the substance to which the muscles are attached by a specialised epithelium ;
- (v.) the Hinge Ligament.

The mass of the shell is further divisible chemically and microscopically into an albuminoid substance called "conchyolin" and crystalline carbonate of lime deposited therein. Römer's careful observations (32) have shown beyond a doubt that there is a sharp separation between these two substances: the conchyolin forming an alveolar framework, in the chambers of which the salts are deposited; the structure of the calcium carbonate being crystalline, its form being determined by that of the spaces in which it is deposited.

The ratio of conchyolin to calcareous salts differs in different parts of the shell. Thus Römer (32) has found in *Margaritana*, the fresh-water pearl-mussel, that the organic substance constitutes 1.47 per cent. by weight of the prismatic substance, but only .64 per cent. of the nacre. This is most interesting as giving support to the theory of the present writer, enunciated below, that the different structures of the different forms of shell-building substances, normal and pathological, are in part a function of the proportions in which these two constituents are secreted by the tissues of the mollusc.

To turn now to the details of the structure of the several constituents of the shell.

(i.) *The Periostracum.*

The origin of the Periostracum can best be understood if we consider first those forms which live in fresh or estuarine water, or are otherwise subjected to conditions which render necessary a thick cuticle-like layer to defend them from the erosive action of organic acids derived from decomposing animal and vegetable matter (*e.g.* the Unionidæ and *Mytilus*). The periostracum in such cases has been described fully by several authors, *e.g.* Biedermann (1), Moynier de Villepoix (28), Tullberg (47), Ehrenbaum (9), Felix Müller (29), Stempell (44), List (27*b*), etc.

In these cases the periostracum is composed of two constituents. The outermost layer is probably formed as a true cuticle directly by transformation or cuticularisation of the outer surfaces of the cells of a specialised epithelium in the inner (axial) face of a deep groove which runs along the mantle-margin, and which has been called by Moynier de Villepoix (28, p. 18) the "fente marginale." This marginal groove divides the mantle-margin into an inner and an outer lobe, the former being pigmented and sensory, the latter being a part of the shell-secreting apparatus. This outer

layer of the periostracum is closely adherent to the specialised epithelium, so that secondary thickening, if it takes place (and my own observations on *Mytilus* and *Modiola* lead me to think that it does so to some extent, a view which is held also by List, 27 b, p. 55), differs from that of all other parts of the shell (including the inner layers of the periostracum) in that it is secreted from outside or centrifugally with respect to the body of the animal and the shell, instead of from inside or centripetally. It would, indeed, seem as though there were morphological grounds for restricting the name periostracum to this particular layer of the outer cuticle-like substance, or, failing that, for introducing a term which would separate it more sharply from the more bulky inner layers. The structural distinctions of this layer are well shown by Römer (32) fig. 25 (*Margaritana*), by Moynier (28) fig. 50 (*Mytilus*), by Tullberg (47) Taf. iv. fig. 3 c, fig. 4 d (*Mytilus*), and by List (27 b) in the Mytilidæ generally.

The greater part of the periostracum, however, is laid down centripetally, layer upon layer, by the epithelium on the outer side of the marginal groove (*i. e.* on the inner side of the outer of the two lobes of the mantle-margin, Tullberg (47) p. 27). It is stratified, and in *Mytilus* contains, near its outer limit, a characteristic layer of large alveoli. The stratifications of this substance have been shown by Römer to correspond to layers of minute alveoli (32, fig. 25).

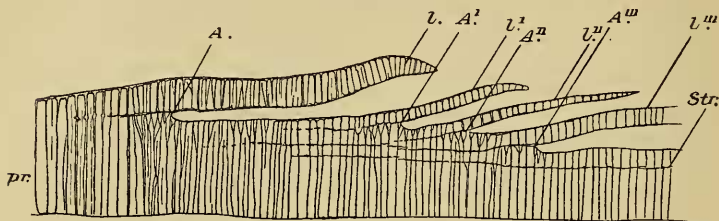
Internally the inner layers of the periostracum pass over into the conchyolin framework of the prismatic layer.

In some forms, *e. g.* *Anodonta*, the distinction between the two constituents of the periostracum are emphasized by the outer layer being much greater in area than the inner ones, and being thrown into folds upon which the inner layers lie unconformably.

The Mother-of-Pearl Oysters (together with such forms as *Ostrea* and *Pecten*) differ from the types to which the above description refers in the fineness of the periostracum and in having much more freely retractile mantle-margins. In sections of the decalcified shells of the Mother-of-Pearl Oysters it is difficult—indeed, I might say impossible—to differentiate the periostracum from the outer layer of the organic basis of the prismatic substance (text-fig. 35, p. 302; see also Pl. XXXIV. fig. 6). Here marginal growth proceeds by a series of steps and retreats, the free mantle-margin being retractile to the edge of the nacre, and being so retracted when the shell closes. Thus, after a new process of the lip has been formed, the mantle-margin is withdrawn, and forms a fresh attachment on the inner surface of the last-formed lip, from which a fresh lip is produced. So the periostracum of lip no. 2 is attached to and apparently a direct continuation of the inner surface of the conchyolin of the prismatic layer of lip no. 1, and constitutes the outer layer of the conchyolin of the prismatic layer of lip no. 2. But Herdman has shown that, difficult as it is to distinguish a separate periostracum in sections of the shell, such a layer, of extreme delicacy, does exist at the

margin, and arises in the normal manner in a marginal groove (Ceylon Report, Part II. Anatomy of Pearl-Oyster, plate viii. fig. 2).

Text-fig. 35.



Margaritifera vulgaris, Persian Gulf. Section through the lip of the shell, after decalcification, showing the successive lappet-like processes of the prismatic substance (l, l', l'', l'''). *pr.*, prismatic substance; *Str.*, stratification of same. *A, A', A'', A'''*, points marking the successive retreats of the secreting margin, which takes place when new lappets are to be formed. At these points the "periostracum" of the new lappet is continuous with, and indistinguishable from, the inner conchyolin-layer of the prismatic substance of the last-formed lappet. Preparation X ($\times 35$), see also Pl. XXXIV. fig. 6.

(ii.) *The Prismatic Substance.*

Reduced to its simplest terms the prismatic layer of the Mother-of-Pearl shell consists of prisms of calcium carbonate perpendicular to the surface of the shell, bounded externally and internally by membranes of conchyolin, which are connected by vertical membranes forming the septa between the prisms (text-figs. 35 and 36; see also Pl. XXXIV. figs. 6, 6 *a*; Pl. XL. fig. 29 *a*). Where interruptions in the continuity of the growth of this layer have occurred, the layer of prisms may be divided by one or more horizontal walls of conchyolin (text-figs. 35, *str.*, and 38, *str.*), which break up the prisms into segments, or which divide the layer into two or more series of prisms. The individual ends of the prisms in one layer do not of necessity coincide with those of the prisms in the next layer, though they frequently do coincide. The septa between the prisms may also show annular thickenings, corresponding to zones of constriction around the prisms (Pl. XXXIV. fig. 6 *a*, *ann.*). Römer has shown (32, p. 35) that the prisms of the pearl-shell (like those of *Pinna*, described by Biedermann (1), p. 9) behave between crossed nicols in the same manner as single crystals. The prisms differ enormously in size, according to the age of the oyster and the conditions under which they are secreted.

There is a sharp line of demarcation between the prismatic and the nacreous layers in *Margaritifera*, the innermost layer of the conchyolin of the former being connected to the outermost layer of that of the latter by a series of fine connectives of conchyolin (Pl. XXXIV. fig. 6 *a*, *con.*), forming a curious alveolar layer.

The prismatic substance is secreted by that part of the epidermis apposed to the inner surface of the shell which is nearest to the margin of the mantle, and it is clear from the rapidity with which the columnar lip and its lappet-like processes are regenerated when injured and are added to in growing young shells that the characters of this layer are associated with relatively rapid secretion. This is significant, in view of the resemblance of this layer to some of the rapidly secreted repair-substances which replace the nacre under certain abnormal conditions (see below).

Römer's work (p. 18) gives an interpretation of the nature of the prisms, which I have found most useful in helping me to interpret my own observations on pearl-formation. According to this hypothesis, which was suggested to Römer by my illustrious teacher, Prof. Bütschli, each prism is an incomplete sphaerocrystal, the growth of which has been arrested in all directions but one, viz. the direction from which the new shell-substance is secreted. Römer says (p. 18):—

“Dass diese Sphärokristalle der einzelnen Prismen so unvollständig ausgebildet sind, rührt daher, dass gleichzeitig und dicht nebeneinander die Anfänge der einzelnen Prismen oder Spärokristalle gebildet wurden, die bald seitlich aufeinander stiessen und sich so gegenseitig in der weiteren Ausbildung hemmten; nur an ihren inneren Enden vermochten sie einseitig weiter zu wachsen”*.

If I may be allowed to state the proposition in slightly different terms, the prismatic shell-substance (and, indeed, if my interpretation of the variations of the nacre, normal and pathological, are correct, the whole of the shell-substance) agrees with a sphaerocrystal in that it is composed of crystalline or crystallised substance which can only grow by the apposition of fresh layers deposited on a single surface, owing to the matter in solution only having access to one surface of the crystalline mass. When this surface is the outer surface of a sphere, a body with more or less of the characters of a sphaerocrystal results (*e.g.* Harting's bodies and Pearls); where it is approximately a plane surface, as in the growth of the Molluscan shell, a structure such as the

* Bütschli in 1908 (6, p. 26) explained his definition of “sphaerocrystals,” more especially with reference to the crystal-like prisms of *Pinna* (and by analogy of *Margaritifera*), as follows:—

“Wie aus den Darlegungen in meinem Werk von 1898 hervorgeht, verstehe ich unter einen solchen nicht ein Aggregat zentrisch angeordneter Kristallnadeln oder Einzelkristalle, was zwar die übliche Anschauung ist, sondern ein einheitliches Kristallgebilde, in welchem die besonderen feinsten Strukturverhältnisse, die auch den gewöhnlichen Kristallen ihre charakteristischen Eigenschaften verleihen, nicht entsprechend einer Axe, sondern um ein Zentrum radiär orientiert sind. Wenn daher der Radius eines solchen Sphärokristalls sehr gross wird, und man ein radiales Stück desselben, weit entfernt von dem Zentrum, herauschneidet—und so verhalten sich etwa die Pinnaprismen—so muss dieses Stück sich natürlich wie ein gewöhnlicher Kristall verhalten; obgleich die von mir gegebene Zurückführung auf einen Sphärokristall mit grossem Radius ganz zutreffend ist.”

Molluscan shell results, the axes of its constituent elements being approximately parallel.

(iii.) *The Nacre.*

This substance, which forms the bulk of the shell, and gives the shells of the genus *Margaritifera* their commercial value as Mother-of-Pearl, and the pearls their beauty, is stratified, and in it the calcium carbonate is divided into extremely minute bodies in the organic network. It is secreted by the outer surface of the mantle and body-wall.

I will not attempt here to review the many writings on the structure of this layer. I can at present add little to the recent work of Römer (32), who has studied its structure and that of its decalcified conchyolin framework very thoroughly. The organic basis which gives it its form, and which retains its iridescence after the calcareous salts have been extracted, consists of a series of parallel lamellæ, of extreme fineness, united to one another at intervals by radial connections, so as to form a series of minute flat or lenticular chambers, separated by organic walls of extreme delicacy. The calcium carbonate appears to be enclosed in these chambers in the form of little polygonal plates or lozenges. This structure is difficult to observe, owing to the distorting effect of the decalcification process, which, owing to the evolution of gas-bubbles, tears some lamellæ apart and forces others tightly together. It becomes much more obvious in some of the abnormal and pathological varieties of nacre described as "repair-substance" below, notably in "granular repair-nacre."

I believe that the lustre (not the iridescence) of mother-of-pearl, and of pearls, is in great measure due to the fact that each of these tiny plates is a minute, biconvex lens; and that the extraordinary and indescribable character of the light reflected from the surface of a fine pearl is in part the cumulative expression of the action of these myriads of little lenses upon the light reflected from the surfaces of calcium carbonate and of conchyolin which underlie them.

(iv.) *Hypostracum, or Muscle-Attachment Substance.*

This curious substance has not secured all the attention it deserves. It has been described by several writers under the names Hypostracum (Thiele, 46), Stäbchenschicht (F. Müller, 29), durchsichtige Substanz (Ehrenbaum, 9; Tullberg, 47).

I retain the name hypostracum, as emphasizing the distinct origin and characters of this layer, and as shorter and more convenient than "durchsichtige Substanz."

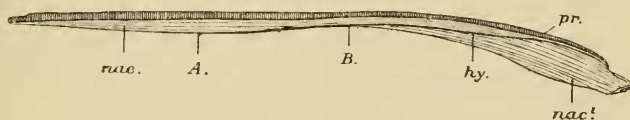
This hypostracum is a fine columnar layer forming the surfaces where the muscles are inserted into the shell (Pl. XXXIV. figs. 5 & 7, *hy.*; text-figs. 36 & 37).

It is more transparent than the nacre—indeed, the iridescence and lustre of the muscle-scar is due to the nacre lying below and

shining through this "durchsichtige Substanz," the substance itself not possessing the structure to which these optical properties are due.

It is composed of columnar or fibrocrystalline needles of carbonate of lime (Stäbchenschicht, Müller), but shows in places, in addition to its columnar structure, a distinct stratification parallel to the surface; this is seen also in the basis which remains on decalcification (Pl. XXXIV. fig. 5). I attribute this stratification to variations in the organic basis, which are probably independent of the form and structure of the crystalline needles. Hypostracum only occurs where the specialised muscle-attachment epithelium is inserted into the shell, and, as the muscles move away from the umbonal region with the growth of the shell, it is quickly covered over by ordinary nacre which is deposited in the wake of the advancing muscle. In a section of the shell from the umbo through the adductor scar the hypostracum layer can be traced across the shell through the nacre from the scar to the umbo, the thickness of the overlying nacre increasing as the umbo is approached. By means of this hypostracum layer, the wandering of the adductor muscle is recorded in the shell-substance (text-fig. 36, *hy.*).

Text-fig. 36.

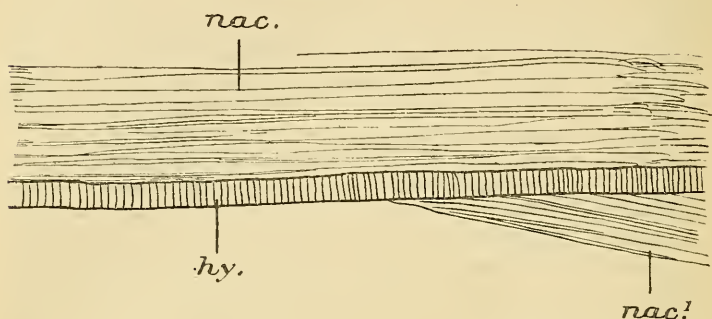


Margaritifera maxima Jameson. A young shell or "chicken shell" from Port Darwin, Northern Territory of Australia (London markets). Section from the umbo to the shell-margin passing through the middle of the adductor impression. *pr.*, prismatic layer; *A-B*, muscle-scar, covered with "Hypostracum"; *hy.*, the hypostracum layer, by means of which the migration of the muscle, from what is now the umbonal region, with the growth of the shell can be traced; *nac.*, nacre of the shell-margin, formed external to the muscle-scar; *nac.!*, nacre of the thick subumbonal region, deposited internally to the hypostracum. Two-thirds of natural size.

In the shell figured, a young example of the large white Australian Mother-of-Pearl shell (*M. maxima* Jameson), the hypostracum is 18–20 μ thick over the muscle-scar, thinning out to 10 μ and then to 4 or 5 μ at the extreme outer edge of the scar, where the muscle has most recently made attachment. As this layer is traced backwards towards the umbo, through the nacre, it is found to get gradually thinner, just as the prismatic substance (which in this shell is about 1 mm. thick in the region of the adductor scar, and in the lip of very old examples of the same species may be 2 or 3 mm. in thickness) is found to get thinner towards the umbonal region. These differences are no doubt associated with the relative ages and sizes of the animal at

the respective periods, and with the relative rapidity of peripheral growth in young and older oysters. Text-fig. 37 shows the hypostracum of the same example (*M. maxima*) enlarged forty times.

Text-fig. 37.



The inner limit of the adductor scar, in the same shell as that shown in text-fig. 36. *hy.*, hypostracum; *nac.*, nacre external to same; *nac.!*, nacre internal to same. $\times 40$.

Exactly the same relations occur in *M. vulgaris*, a section through the umbonal side of the adductor scar of which is shown in Pl. XXXIV. fig. 7. But in this example, an old thick Lingah shell from the Persian Gulf, in which, in all probability, peripheral growth, and consequently the wandering of the muscle, had ceased, the hypostracum is thicker, measuring $130\ \mu$ in thickness.

The hypostracum undergoes but little secondary thickening, compared with the nacre. Hence, in thick massive shells like *M. maxima*, where the newly forming nacre in the umbonal region and also towards the lip and around the muscle-scar outstrips the hypostracum in development, the muscle-scar is the thinnest part of the entire shell, except the extreme edge. This is well seen in text-fig. 36, in which figure the area between A and B represents the muscle-impression, but it is even more obvious in older thicker shells. Some interstratification of hypostracum and nacre occurs at the borders of the muscle-scars, where changes in the outline of the muscle have taken place. This is figured by Tullberg in *Mytilus* (47, Taf. v. fig. 2). The same is shown for *Margaritifera vulgaris* at *hy.*!, *hy.*!, in Pl. XXXIV. fig. 7. Felix Müller (29, Taf. xxix. fig. 13 b) shows the lateral transition of this substance into nacre. I have observed the same thing in some of the "Muscle-Pearls" described below.

When decalcified the hypostracum leaves behind it an organic basis, which is somewhat different from the conchyolin of the rest of the shell in its reaction towards stains. This is of interest in connection with the view generally held that this layer, unlike the other calcareous parts of the shell, which are probably

due to simple secretion, arises by a gradual transformation into shell-substance of the outer regions of the specialised epidermal cells which underlie it, *i. e.* in the same manner as the Crustacean carapace and the outermost layer of the periostracum. It shows a well-marked striation perpendicular to the surface, the striae no doubt corresponding to the outlines of the spaces which were occupied by the needle-like fibrocrystalline bodies of calcium carbonate, and also at times indistinct lines parallel to the surface (Pl. XXXIV, fig. 5). In sections in the plane parallel to the surface this substance shows an alveolar structure. It sometimes shows a tendency to break up into segments, corresponding to the underlying epithelium-cells (Pl. XXXIV, fig. 5, *hy.*).

(v.) Hinge-Ligament.

I do not propose to discuss the hinge-ligament here, as it has not the same direct bearing on the question of pearl-formation as the above layers, though leathery pearls, composed of this substance, are sometimes found (e. g. in *M. maxima* in Australia).

(10) THE SHELL-SECRETING EPITHELIA.

The ordinary shell-secreting epidermis of *M. vulgaris*, so far as I have been able to study it in the unsatisfactory material available, consists of columnar or tessellated cells (Pl. XXXV. fig. 8, *ep.*; fig. 9, *o.ep.*; Pl. XXXVI. fig. 11, *o.ep.*; Pl. XLI. fig. 33, *ep.*), brick-shaped or palisade-like, according to the degree of contraction, in sections perpendicular to the surface, with a certain and variable number of goblet- and gland-cells. The nuclei of the epidermal cells are oval or spindle-shaped. These cells are attached to the subjacent tissues by a basement-membrane of delicate fibrillæ which distinctly marks the boundary between the epidermal epithelium and the subjacent tissues. Beneath this epidermis is a characteristic granular parenchyma (Pl. XXXV. figs. 8 & 9; Pl. XXXVI. figs. 10 & 11; Pl. XXXVII. fig. 14; Pl. XLI. fig. 33, *par.*), which contains a great variety of elements, some being comparable to the "Rundzellen" and "Langer'schen Blasen" described by List (27 *b*) for the Mytilidæ, some being dark-staining, apparently glandular elements that open out between the epithelial cells (fig. 33, *gl.*). As observed by List (27 *b*), this epithelium and the underlying tissues are excessively variable in their characters.

Over the surface of the muscle-attachment the epidermis is different (Pl. XXXIII. figs. 4, 4 *a*; Pl. XXXV. fig. 8, *m.ep.*). Here it consists of columnar cells, usually about 10–12 μ long and 2–4 μ broad, which pass over basally without any distinct dividing-line into the muscle-fibres. Whether the transition is direct, or whether in fact a connective-tissue junction is present, cannot be determined from the available preparations of *Margaritifera vulgaris*; but in *Mytilus edulis* there is a distinct connective-tissue layer (fig. 5, *c.t.*), the fibres of which,

continuous with the bases of the epidermal cells, are attached to the ends of the muscle-fibres (*musc.*), which may be produced out into tails. Tullberg recognised that such a junction was present. In *Margaritifera vulgaris* the attachment epithelium-cells may have one or several tails, probably also of connective-tissue character, passing over into as many muscle-fibres.

Distally these epidermal cells broaden out somewhat, ending in a clean-cut surface, which may be represented in section by a clear zone forming a slightly acute angle with the sides. The nuclei, which are oval and about 3-4 μ long, are situated in the middle of their length.

It is possible to make out, in some cases, a striation of these cells in the direction of their long axes. Occasionally the distal surface is raised into processes and papillæ, but this may well be a result of imperfect fixation of the tissues.

These cells stand out as stiff, independent, almost bristle-like entities, and are probably hard and tendinous in character. They are frequently preserved in old preparations in which all traces of the structure of the ordinary epithelia have disappeared. In some cases they seem to have been drawn out in the fixing process; thus the longest cell shown in Pl. XXXIII. fig. 4 *a* measured 26 μ . It seems possible that in the shrinkage consequent upon fixation the majority of the elements here had broken away from the shell, but that this particular cell had remained attached and was consequently fixed in a state of extension. This figure shows that the connective-tissue elements extend up between the bases of these cells.

This epidermis is very closely adherent to the specialised shell-layer (hypostracum) to which it is attached, and the connection seems to be between the cells and the organic basis of the shell. Thus, in decalcifying a piece of the shell of *Mytilus* with the adductor muscle attached, the hypostracum remained adherent to the epithelium and tore away from the rest of the shell (Pl. XXXIV. fig. 5).

(11) SHELL-SECRETION.

I will not attempt to survey the writings of previous investigators on this subject. This has been ably done by Stempell (45), whose review contains a full and lucid discussion of the question.

The general trend of opinion now seems to favour the theory dating back to Reaumer, 1709 (31), and held by Tullberg, Ehrenbaum, Moynier de Villepoix, and the majority of recent French and German investigators, that the shell (except the outermost layer of the periostracum and the hypostracum) is formed from a fluid secretion, rather than the theory specially associated with Huxley's name (24) that the shell is derived from a succession of fully developed skins or cuticles, shed as membranes by the underlying epidermis.

The outermost layer of the periostracum (which in *Margaritifera* is a negligible quantity) and the hypostracum probably arise by direct transformation of the outermost portions of specialised epidermal cells, and on this account it may prove necessary to draw a sharper morphological distinction between them and the rest of the shell than has hitherto been done*.

The prismatic layer and the nacre, together with the inner layers of the periostracum, more probably arise as a secretion which first hardens into a membrane *in situ*, and then forms the delicate skin which Huxley observed between the mantle and the shell in the freshwater mussels.

It would appear that the lime-salts and albuminous fluid which hardens to form the conchyolin are independent of each other, and may be secreted in varying proportions. Where these two constituents are secreted under circumstances which inhibit the control of the shell-secreting epidermis, or where the secretion takes place so copiously and rapidly that the epidermis is unable to regulate the deposition (as in the pathological cases described below), lime-salts are precipitated in a columnar form, much as in Harting's bodies, and, concurrently with this, the albuminous fluid is transformed into an insoluble substance resembling conchyolin. The process of shell-secretion at the rapidly growing edge of the shell resulting in the formation of the prismatic layer—which in *Margaritifera vulgaris* measures as much as 1 mm. or more in thickness—is probably in some degree analogous to the process of secretion of repair-substance, the epithelium exercising comparatively little control over the arrangement of the elements.

But in the case of the nacre it is different. Here the epithelium seems to exert a definite and very strict selective influence resulting in the finely stratified and chambered structure which can, I think, best be interpreted as arising from rhythmically intermittent secretory action on the part of the controlling epidermis. Any disturbance of the normal rhythm of this secretion, *e. g.* the stimulation of an intrusive particle between shell and epidermis, results in the formation of the irregular substances described below, such as granular repair-nacre, the several varieties of columnar repair-substance, or the amorphous non-calcified substance.

It would thus seem as though the structure of the shell-substance, and its variations, normal and pathological, could be expressed in terms of the proportions of lime-salts and organic

* The difference between the outermost layer of the periostracum and the hypostracum on the one hand, and the remainder of the shell on the other, the former parts arising by direct cell-transformation or cuticularisation of cell-protoplasm, the latter as a secretion poured out by the cells, suggests a line of inquiry that might yield interesting results. Can these two constituents of the shell be separated morphologically and phylogenetically, and, if so, can the former be regarded as in any sense homologous with the cuticular exoskeleton of an ancestor common to Mollusca and Arthropoda, the latter being a subsequent addition peculiar to the Mollusca, associated with their more sedentary modes of life, which has now, for all practical purposes, replaced the more strictly cuticular element as an exoskeleton?

salts secreted and of the periodicity of the secretion as determined by the control, or loss of control, of the secreting epidermis.

In fact, if my interpretation is correct, the processes involved in the building of the shell are the usual chemico-physical ones which govern crystallisation in colloidal media* controlled and limited by the time-factor which is a function of the activity of the living cells.

It is less easy to imagine the conditions which determine the transformation of the fluid albuminous secretion into the leathery conchyolin. One is naturally tempted to postulate a chemical transformation as a direct or indirect result of the action of nascent CaCO_3 , as in the case of the calcoglobulin in Harting's bodies (12); but the formation of this substance apart from the lime-salts, *e.g.* in the inner layers of the periostracum and in amorphous repair-substance, and in the case of shells grown in lime-free media (Moynier de Villepoix, 28, p. 122), seems to negative this; and it may well be that this change to an insoluble albuminoid is directly brought about by the action of the secreting cells themselves, or follows from the chemical composition of the secretion as shed.

(12) ABNORMAL AND PATHOLOGICAL PHASES OF THE SHELL-SUBSTANCE.

For a study of the beginnings of Ceylon pearls, a consideration of the variations in the shell-substance, when it is secreted under abnormal conditions, either on the surface of the shell or of a growing pearl, is of importance.

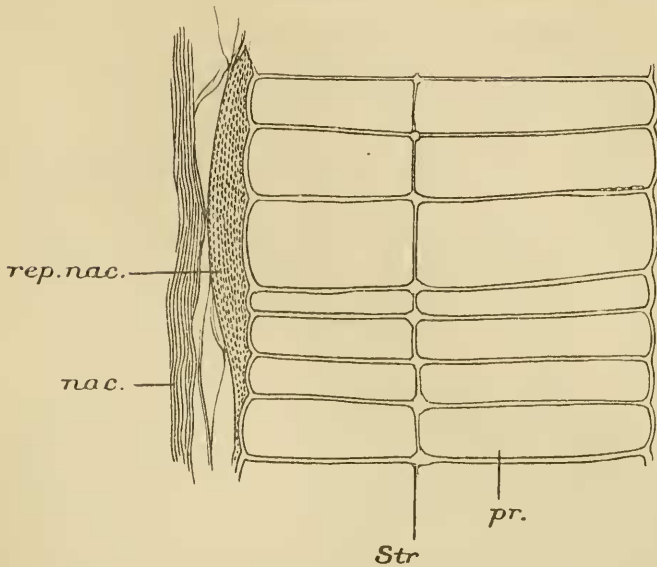
Where the normal rhythm of the process of shell-secretion is interrupted, *e.g.* by injury to the shell, or the intrusion between the epithelium and the nacre of a foreign particle or by other disturbances less easy to explain, certain irregularities in the process of secretion occur, resulting in an altered product.

In the simplest case such a disturbance results in a modification producing a granular appearance of the conchyolin-layers of the nacre. This modified substance I propose to call "granular repair-nacre." In sections made through this substance, after decalcification, the normal stratification is obscured by a highly granular appearance which seems to be due to an infinite number of connections between the successive conchyolin-layers resulting in a distinctly alveolar membrane. This is shown in text-fig. 38 (*rep.nac.*), which is taken from an artificial "blister" produced by the writer in *Margaritifera margaritifera* after the "Linnaeus" method, in British New Guinea in 1899. The foreign body was inserted near the mantle-margin, and the mantle secreted first a double layer of the prismatic substance,

* Biedermann (2), p. 171, recognises that the structure of the shell is essentially reducible to crystallisation processes, the influence of the cells being limited to the composition of the fluid, and perhaps the orientation of the primary centres of crystallisation. But I would add to these influences the periodicity of their action.

and then nacre, which, at places, showed the characters of "granular repair-nacre."

Text-fig. 38.



Margaritifera margaritifera Linnaeus (Black-lipped Mother-of-Pearl Oyster). Part of an artificially produced blister. *pr.*, prismatic layer; *Str.*, horizontal dividing membrane of conchyolin in same; *nac.*, nacre; *rep.nac.*, granular repair-nacre. (Preparation VIII.)

Pl. XL. fig. 29, from the "repair-membrane" formed by *M. vulgaris* over a hole in the shell (umbonal region), shows the same substance at *tr.* passing over on the one hand into columnar repair-substance, on the other into nacre. The same substance is seen at *tr.*

Pl. XLI. fig. 30 (*rep.nac.*) shows the same substance, secreted at the point of junction of two pearls (from one of Dr. Kelaart's specimens of *M. vulgaris*). Here it was secreted as a result of disturbances following upon the fusion of the two pearls and the absorption or calcification of the intervening tissues. The granular repair-nacre in the preparation shows in places a distinctly columnar structure, indicating a transition to the columnar repair-substance; such a transition is still more obvious in Pl. XL. fig. 29.

The same granular repair-nacre is seen in Pl. XXXV. fig. 9, Pl. XXXVII. fig. 15, and Pl. XXXVIII. fig. 18, surrounding the central cavities of "muscle-pearls," where unduly rapid secretion might well be expected, and in Pl. XXXIX. fig. 23, where a hypostracum-pearl ("calcospherule," Herdman) is in process

of being coated over with nacre. The same substance is well shown in Pl. XLI. fig. 35 and Pl. XLIII. fig. 43 (*gr.*). In the last-named case it is seen to pass over on the one hand into nacre, on the other into columnar and amorphous repair-substances.

The next form of repair-substance is much more variable, and occurs in several distinct, though intergrading forms. I propose to call this "columnar repair-substance," in view of the calcium carbonate being crystallised in columns.

Columnar substance resembles, more or less, the prismatic layer of the shell—indeed, it is probable that Rubbel (33, p. 171) had a substance analogous to this columnar substance before him when he stated that the outer epithelium of the mantle of *Margaritana* is capable, in repairing the shell, of producing the prismatic substance which is normally only the product of the mantle-margin. (In the same way, he treats as "periostacum" the non-calcified material secreted under similar conditions, which I describe below as "amorphous repair-substance".) *

In its simplest form columnar repair-substance consists of parallel needle-like rods of carbonate of lime (which Steinmann (43), speaking of Harting's bodies, has aptly called "fibro-crystalline") deposited in an organic conchyolin-matrix, which, when the calcium carbonate is removed by acids, and a section is cut at right angles to the surface, presents a palisade-like appearance, due to the septa of conchyolin between the calcareous rods (Pl. XL. fig. 29; Pl. XLI. fig. 30, *col.*). In horizontal section this conchyolin has a honeycomb-like structure.

All kinds of variations occur in the coarseness or fineness of the calcareous elements and the organic framework.

This substance is frequently formed on the surface of the shell or of a pearl when disturbances arise in the rhythm of shell-secretion. In Pl. XL. fig. 29 it is seen in the repair-membrane formed over an injury caused to the shell by a boring parasite. In Pl. XLI. fig. 30 it is seen (*col.*) in the angle between the surfaces of two pearls which have become secondarily attached together.

Pl. XLI. fig. 31 shows the same substance developed under conditions similar to those existing in fig. 30. This figure is a drawing of a section through the suture between two pearls which have become secondarily fused together. The pearls themselves, with the intervening suture, are shown in Pl. XLIV. fig. 49; the end of the suture, where the curvatures of the two pearls diverge, in fig. 31. In the entire object, examined in oil of cloves (fig. 49), the suture was represented by a yellowish-brown line, the colour being due to the dead remains of the cellular membrane which originally separated the two pearls.

* While these substances are perhaps not strictly separable respectively on chemical and physiological grounds, I think it is well on morphological and pathological grounds to emphasize the distinction.

The membrane consisted of the lining epithelia of the two sacs, and a layer of parenchymatous tissue between these two epithelia.

The epithelia, and even the individual cells of the parenchyma, can be detected in some places (Pl. XLI. fig. 32).

If we try to trace the steps resulting in the condition figured on Pl. XLIV. fig. 49 and on Pl. XLI. fig. 31 (*i.e.* to survey the story of the formation of a double pearl), we may assume that they were as follows. As the two neighbouring pearls, each enclosed in a sac, grew in size, by the addition of fresh layers, they exerted a pressure on the intervening tissues, resulting in reduced circulation and consequent malnutrition which began at the first point of contact and extended outwards. Thus the contiguous surfaces tended to become flattened (fig. 49), and the intervening tissue, consisting of the epithelia of the two pearlsacs and a small amount of connective-tissue between them, finally ceased to be functional, died, and was preserved as a yellow membrane (Pl. XLI. fig. 32). At the periphery of the area of contact, where the curvatures of the two pearls diverged and were separated by a wedge-shaped plug of tissue, nacre-secretion continued longer, the last efforts of the epithelia being represented by *nac.* and *nac.'* in fig. 31. Finally, the epithelium ceased to control the deposition of its secretion, and, with the shrinkage of the atrophied tissues a space occurred on each side between the nacre and the epithelium, into which an extravasation of organic matter and salts occurred. The salts precipitated themselves in the form of columns or raphides with their bases apparently in or on the epithelia, and concurrently with this precipitation the soluble organic substance became converted into the conchyolin framework between the prisms, analogous to the "calcoglobin" framework of Harting's bodies, derived from egg-albumen when calcium carbonate is precipitated in it. Lastly, the epithelia and intervening connective-tissue died and probably underwent irregular calcification, breaking away from the still functional tissues and becoming incorporated in the substance of the pearl. The still functional tissues now formed a single sac surrounding the two pearls, and quickly enveloped them both in a common nacreous covering.

Similar processes can be postulated to account for the condition shown in Pl. XLI. fig. 30. Here, between the curvatures of the surfaces of the two contiguous pearls, there was a triangular plug of tissue, which for some time remained attached to the degenerated membrane which separated the pearls. Its epithelium gave rise before it broke away to granular repair-nacre (*rep.nac.*) on the right, where the disturbance was presumably least, and to a small amount of columnar repair-substance (*col.*) on the left. Then it broke away from the degenerated and dead membrane between the pearls and retreated rapidly, exuding as it went the albuminous fluid, which, being secreted at a much greater rate than the lime-salts, was practically devoid of lime

and formed coarsely stratified amorphous substance (*am.*), broken by cleft-like cavities. Later on, when the retreat of the plug of tissue was less rapid, this amorphous substance passed over into columnar substance (*tr.*) and granular repair-nacre (*tr.*'), and finally gave place to the nacre (*nac.*') of the common investment of the compound pearl.

The columnar repair-substance varies enormously, and passes over imperceptibly into "amorphous substance" or lime-free conchyolin, granular repair-nacre, ordinary nacre, and the prismatic substance of the shell. For example, the repair-membrane, a part of which is shown in Pl. XL. fig. 29, showed an immense number of variations from place to place. In some parts a second layer of amorphous substance was interpolated between the columnar layers; in others the columnar substance passed over into a coarsely alveolar substance with irregular cavities, some of which penetrated into the amorphous substance. In yet other spots the amorphous substance passed over through granular repair-substance into nacre.

Columnar substance is frequently stratified, consisting of a number of consecutive layers. This is seen at *col.*' in the repair-membrane figured at fig. 29. It is also shown in the pseudo-nucleus of the pearl shown on Pl. XL. fig. 28 and Pl. XLIV. fig. 49, and in the pearls from the Persian Gulf in Pl. XII. figs. 33 & 34. In the former of these last-named instances it occurs immediately around the central cavity, in the latter case interstratified and intergrading with the nacre.

In Pl. XLIII. fig. 43 (*col.*) it is seen passing over on the one hand into granular repair-nacre, on the other into amorphous repair-substance. The same stratified columnar substance is well shown in Pl. XLV. fig. 51, where it forms a curious flaw running through the substance of a pearl.

Apart from this direct stratification, the columnar repair-substance may have an internal alveolar structure such as is shown in Pl. XLII. figs. 36, 37, & 38. Figs. 40-42 on the same Plate, taken from the pearl shown in Pl. XLVI. fig. 57 (a brown pearl composed of prismatic shell-substance), show the transition from amorphous repair-substance to columnar repair-substance (figs. 41, 42, *col.*), and from the latter to the prismatic layer of the shell (fig. 42, *pr.*).

The third variety of repair-substance I call amorphous repair-substance. In its typical form this substance is seen at *am.* in Pl. XL. fig. 29, where it is obviously the result of the first effort of the mollusc to close the injury to the shell, and in Pl. XLI. fig. 30, where it is the product of a fully functional epithelium, retreating rapidly and leaving its secretion in its wake. It shows little or no structure under ordinary magnifications, but is usually faintly stratified. It may contain cavities, arranged in rows parallel to the secreting-surface, and with at times also a radial arrangement. These cavities typically contain carbonate of lime. Pl. XL. fig. 29 *a* (*Margaritifera vulgaris*, Lingah Shell, Persian

Gulf) shows this substance formed as the first step in the development of a new layer of prismatic substance to cover over the tube of the worm *Leucodore*, which has entered between the mantle-margin and the shell, as is its wont. Here the mantle-margin, reacting to the stimulation of the parasite, has retreated and secreted a new "lip" to exclude it. This lip, like the normal lip, consists of the prismatic layer of the shell, but the irregularly secreted first layers of it consist of amorphous substance, containing alveoli in which a scanty supply of calcium carbonate was deposited.

The amorphous substance frequently occurs in the centres and around the central cavities of pearls, where it doubtless represents the first matter which the mollusc shed into the cavity. It probably corresponds to the "Theile des Schalenepidermis" recognised by von Hessling (18, p. 313) in the nuclei of pearls, and the "Kern von Chitinsubstanz" referred to by Pagenstecher (30, p. 502), and perhaps to the "Gelbbrauner Substanz" of Rubbel (34, p. 412).

The amorphous substance shows little receptivity to stains. It passes over sometimes into columnar substance (Pl. XLI. fig. 30, *tr.*), sometimes into granular repair-substance (Pl. XL. fig. 29, *tr.*; *tr.*; Pl. XLI. fig. 30, *tr.*). It also sometimes intergrades with a substance resembling the prismatic layer (Pl. XLII. fig. 41). Similar intergradations with prismatic substance were shown in some parts of the preparation from which Pl. XL. fig. 29*a* is drawn. Pl. XLII. figs. 40-42 are of interest as showing all manners of intergradations between amorphous, columnar, and prismatic substances, the different structures shown being apparently mainly dependent upon the proportions of calcium carbonate present. Thus we have in this pearl, which, owing to the impermeability of the amorphous substance, was imperfectly decalcified, tracing the layers from inside outwards: (1) a plug of nuclear matter of doubtful origin containing well-marked crystals (fig. 40, *nu.*)—these are true crystals (rhombohedral); (2) a layer of amorphous substance, passing over into typical simple columnar repair-substance (fig. 40, *col.*); (3) numerous layers of amorphous substance (figs. 40, 41, *am.*), some layers being quite lime-free, some having scattered alveoli containing calcium carbonate, some showing their cavities in radial rows, leading up, by transitions, to regular columnar substance (figs. 41, 42, *col.*), which differs from the prismatic substance proper (fig. 42, *pr.*) only in the smaller diameters of its constituent elements, a difference which, in view of the variability of the sizes of the prisms in the shell itself, is comparatively unimportant.

Again, the transition from the abnormal repair-substances to nacre in the pearl shown in Pl. XLV. fig. 52, *col.*, and Pl. XLII. fig. 36, *col.*, is equally striking. This is shown in detail in Pl. XLIII. fig. 43. At *nu.* is the outer wall of the sphaerocrystalline or columnar pseudo-nucleus of the pearl. At *nac.* is

shown the normal nacre of the pearl. The first-formed layers of nacre are incomplete, passing over into this area of repair-substance, and all stages of transition may be seen, corresponding to the gradually increasing control exercised by the secreting epithelium.

At first, amorphous substance (*am.*), alveolar in places, was secreted, no doubt with irregular crystallised bodies in the alveoli, some of which are actually preserved in the preparation, owing to incomplete decalcification. Peripherally this gave place to columnar substance (*col.*), which acquired a finely alveolar structure, and passed over, through granular repair-nacre (*gr.*), into normal nacre (*nac.*'), the layers of the conchyolin of which gradually merge into the horizontal markings of the granular substance. The amorphous substance in this preparation varies from layer to layer in the degree to which it is alveolar; at some places it might better be described as coarsely columnar substance.

Amorphous substance seems to be the first product where the shell is perforated and the mantle makes a sudden effort to close an opening to the exterior. In such cases it may be secreted so copiously that a tough leathery skin results, with little or no lime-salts in it (Pl. XL. fig. 29, *am.*). It is likewise secreted in layers when a break occurs in the nacre-secretion of a pearl or of the shell, owing to a pathological extravasation of cellular matter (Pl. XXXVIII. fig. 17, *am.*, *am.*'). These facts suggest that the organic basis of the shell is the constituent the secretion of which varies in quantity, the secreting-tissues (perhaps the granular subepithelial parenchyma in *Margaritifera*) containing a reserve of this material which can be poured out profusely when the shell is injured. It would seem that the lime-salts, on the other hand, are secreted more regularly, so that the mechanism for furnishing these cannot keep pace with that which yields the organic substance when the latter is called upon to make a special effort to repair damage. The resemblance of the inner layers of the periostracum (in forms with a thick periostracum) to amorphous repair-substance may perhaps be explained by postulating the absence or inhibition of the lime-secreting mechanism in the underlying tissues.

That the secretion of calcium carbonate could not keep pace with that of the organic substance, when the latter is produced in large quantities, is easy to understand in view of the very small proportion of CaO in the blood of Mollusca, and indeed of all invertebrates that have been investigated. According to Griffiths (quoted by Bütschli, 6, p. 62), the CaO in the blood of a number of bivalves examined varied from 0.032 per cent. in *Anodonta* to 0.067 per cent. in *Mytilus*.

Amorphous substance is seen in the pseudo-nuclei of pearls in Pl. XXXIX. figs. 20 & 21, and Pl. XL. figs. 24, 26, & 27; figs. 20, 24, & 27 showing particularly well its continuity and intergradation with the organic basis of the columnar repair-substance. In the centre of a pearl it may contain, in addition to the

central cavity, secondary cavities in its substance, in which organic particles are lodged (fig. 20).

The variations of coarsely alveolar structure which amorphous substance shows (*e. g.* Pl. XL. fig. 29 *a* and Pl. XLII. figs. 40 & 41) recall those structures which Bütschli (6, Taf. iii. figg. 20–33) describes in the sphaerocrystals of (?) Trydimite formed when the siliceous concretionary substance of the Bamboo (known as Tabaxir or Tabasheer) is heated; this structure is probably in great measure the expression of the physical conditions (surface tension, etc.) which prevail when two substances in solution or in a colloidal state separate from one another to form a sphaerocrystalline mass.

Pl. XLII. figs. 37 & 39 are of interest as showing another variation of the nacre, in the direction of columnar substance. In this variety of nacre, the conchyolin-layers are connected by a number of thickened junctions, which tend to occur in groups and which are arranged in radial rows. In surface view these junctions appear as groups of dark spots on the conchyolin-layers; in radial section they are as shown in fig. 37, and can also be seen in Pl. XXXVI. fig. 13. These junctions seem to be thickenings of the walls which normally connect the several conchyolin-layers of the nacre to one another; they may, in fact, be regarded as local exaggerations of the condition described as “granular repair-nacre.”

It is interesting to note the peculiar manner in which these repair-substances occur in Japanese “Culture Pearls.” This name was given by the late Professor Mitsukuri (27 *c*, pp. 283–4, pl. xi. fig. 1) to pearl-like bodies—“blisters,” as they would be called on the Australian fisheries—which are artificially produced in the Japanese Pearl-Oyster, *Margaritifera martensii** Dunker.

The production of these “Culture Pearls” is an extensive industry supporting about 100 persons, and is carried on by Mr. Mikimoto on leased areas of sea-bottom in the Bay of Agu, Shima Province, on lines originally suggested by Prof. Mitsukuri in 1890. It has been going as a commercial success since 1898, when the first crop of “Culture Pearls” was marketed. In 1905 the number of oysters operated on per year was from 250,000 to 300,000.

The process, which is protected by patents, is analogous to that adopted by the Chinese in the production of “Buddha Pearls” in the fresh-water mussel, *Dipsas plicatus*, and to the method discovered by Linnæus in the 18th century (see Herdman, 16 *a*), and consists in the introduction between the shell and the mantle † of a bead of nacre, which in due course (the time allowed in Japan is four years) becomes thickly coated over with nacre,

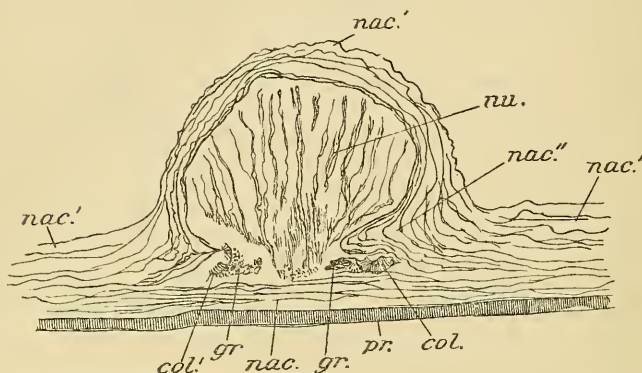
* This mollusc is regarded by some naturalists as a local race of *M. vulgaris*, to which it is undoubtedly very closely related. Whether it be called *M. martensii* or *M. vulgaris* var. *martensii* is largely a matter of individual taste.

† This is apparently done *via* the edge of the shell and not by drilling as in the Linnæus process.

forming a hemispherical, or sometimes rather more than hemispherical pearl-like excrescence, attached to the shell by its base. These "Culture Pearls" are produced in large numbers, and find a ready market for purposes for which "half-pearls" are used. They are now familiar objects in Europe*.

Text-figure 39 is a section of a Japanese "Culture Pearl," which I purchased in London, while still attached to the shell, and decalcified.

Text-fig. 39.



Section through a decalcified Japanese "Culture Pearl" still attached to the shell. *nu.*, the artificial "nucleus," a bead of nacre, the laminae of the nacre being cut transversely; *pr.*, prismatic layer; *nac.*, original nacreous lining, which existed before the nucleus was introduced; *nac.'*, more recent nacre, lining the shell and extending over the "nucleus" to form the "Culture Pearl," secreted after the introduction of the nucleus; *nac.'*, nacreous layers where the lining of the shell is carried over the nucleus; *col., col.'*, repair-substance secreted in a zone around the point of contact between nucleus and shell, where the deposition of the shell-substance was not controlled by the mantle; *gr.*, granular matter, perhaps of foreign origin or of the nature of amorphous substance. $\times 10$.

The "nucleus" has been very skilfully introduced, so that there is practically no trace of "dirt" between it and the nacreous layer with which it is invested, as is so often the case in the "blisters" which have been produced by naturalists and experimenters from time to time. Moreover, the disturbance of the normal functions of the mantle has been so slight that, in the

* Needless to say, these bodies are not "Pearls," biologically speaking, but belong to the class of structures to which I have applied the name "blisters," familiar on the Australian Fisheries and in the Trade. Various naturalists have produced such bodies from time to time. I have recently seen some very beautiful ones produced in *Margaritifera maxima*, and I myself produced some presentable ones in *Margaritifera margaritifera* in Papua in 1899-1900. But although attempts have been made, and are still being made, to do this on a commercial scale, I am not aware that commercial success has yet been achieved anywhere else than in Japan; indeed, I think that the combination of circumstances which has led to the success of the Japanese enterprise—viz., skill, patience, and intelligence, backed by the best scientific advice and supported by cheap labour—has generally been lacking in other ventures. I may add that the price that could be obtained for the best of these gems is insignificant compared with the value of a real pearl of like size. No

particular sections that I examined, there was a marked absence even of the repair-substances. But in the zone immediately around the point of contact between nucleus and shell, where, when the nucleus was introduced, the epithelium of the mantle was presumably unable to fit closely against the surfaces, it is otherwise. Here, on examining the whole "Pearl" as a transparent object after decalcification, an opaque ring or zone was distinctly visible. This was due to the presence of granular matter, perhaps derived from the exterior, perhaps from the tissues of the animal (text-figs. 39 & 40 B, *gr.*), and to very irregular columnar and amorphous repair-substance (*col.*, *col.'*). This columnar substance is shown in greater detail in text-fig. 40, A & B, corresponding respectively to *col.* and *col.'* in text-fig. 39.

In text-fig. 40, A, in the niche between the nucleus and the shell, where the mantle-epithelium could not reach, we see the product of its secretion consolidated away from the influence of the epithelium. Here the columns, instead of forming the characteristic palisade-like structure, with their long axes perpendicular to the secreting surface, are arranged in groups suggesting incomplete spherocrystals.

The curious fan-like arrangement which the columns take on in text-fig. 40, B, suggests that the repair-substance arose through an extravasation of the shell-forming fluids at the point *x*, the layer *nac.*' representing the first normal nacre, secreted by the mantle when it occupied that position, the irregular columnar and amorphous substance being due to the consolidation of the secretion which filled the space, triangular in section, which lay between the nucleus (*nu.*), the shell (*nac.*), and the mantle; the position occupied by the last named being represented by the layer of nacre marked *nac'*.

(13) VARIETIES OF CEYLON PEARLS.

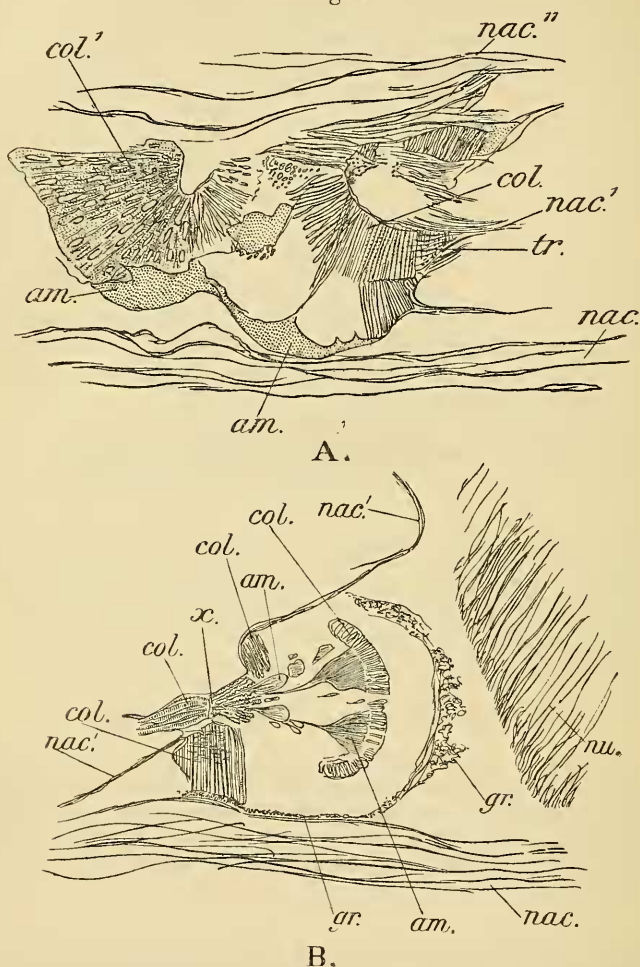
Pending a classification based on the nature of the causes which give rise to the formation of the pearl-sac, I propose, following Herdman, to separate the pearls which I have examined in or from the Ceylon Pearl-Oyster into two main groups, accordingly as they typically occur clustered in the neighbourhoods

really satisfactory proof has ever been given that free spherical "pearls" can be produced in this way, though Prof. Mitsukuri (*l.c.*) says that there are some hopes that this will be done. There is no theoretical reason why a modification of the Japanese or Linnean operation should not be devised which would achieve this end—indeed, there is some reason to think that Linnaeus actually did produce some round "pearls" and not only "blisters." But such bodies, if produced, would not be "pearls" in the strict biological sense, though it is quite likely that they would be marketed as such in quantities before the difference was detected.

Since writing the above, I have been informed by Mr. Toyozo Kobayashi, Professor at the Tokyo Higher Technological College, who is associated with Mr. Mikimoto in his enterprise, that perfectly free "pearls" have been produced by these methods within the last two years, but so far only exceptionally, and on a scale so small as not to be applicable commercially.

of the muscular insertions or singly in the non-muscular parts of the body-wall and mantle.

Text-fig. 40.



- A. The irregular columnar and amorphous substances, shown at *col.* in text-figure 39. *nac.*, nacreous lining of the shell; *nac.*, nacre deposited shortly after introduction of nucleus, passing over into the repair-substance (*tr.*); *nac.*, nacre continuous with the layers investing the nucleus; *col.*, *col.*, columnar repair-substance; *am.*, amorphous repair-substance. $\times 35$.
- B. The fan-shaped mass of repair-substance, shown at *col.* in text-figure 39. *nu.*, the introduced "nucleus"; *nac.*, the original nacreous lining of the shell; *nac.*, the first layers of nacre, separated after the introduction of the nucleus; *col.*, columnar repair-substance; *am.*, amorphous repair-substance; *gr.*, granular matter, perhaps of extraneous origin. $\times 100$.

I adopt Professor Herdman's term "Muscle-Pearls" for the former class, while for the latter category I propose the name "Parenchyma-Pearls"*, because they occur typically in the parenchymatous subepidermal tissues of the non-muscular parts of the body-wall and mantle, or, by secondary displacement, in the more deeply seated soft tissues.

This group corresponds, I think, to Herdman's "Cyst-Pearls," but I prefer not to adopt the latter name, as, if the word "cyst" refers to the encysted Cestode, which Herdman associated with pearl-production, I have been unable to trace the connection between it and the pearl; while if it refers to the pearl-sac or "cyst," this is found around all pearls, including muscle-pearls. There is some reason for believing that some parenchyma-pearls arise from causes different from those that lead to the formation of muscle-pearls, and, indeed, it is quite possible that parenchyma-pearls have several modes of origin, as Herdman believes; but, on the other hand, their differences may be due in great measure to the different parts of the tissues in which they originate, and it is certainly quite impossible, in many cases, to say, from the structure of a pearl and of its nucleus and pseudo-nucleus, whether it is a "muscle-pearl" or a "parenchyma-pearl." With regard to Herdman's "Ampullar pearls," I cannot regard this group as of equal value to the above two classes, as, in my experience, so far as it goes, the "Ampulla" is of secondary origin, due to the absorption of the tissues intervening between the pearl and the shell, and to the epithelium of the pearl-sac and that of the outer face of the mantle thus becoming continuous.

Before going further I had better explain a term that I am introducing into this paper. I am restricting the word "Nucleus," as applied to the body found in the centre of a pearl, to those bodies which appear to be either of foreign origin or derived from the pearl-oyster otherwise than through the agency of the shell-secreting mechanism. To the bodies formed by the shell- and pearl-secreting mechanism, composed, as a rule, of different kinds of repair-substance (bodies which have no doubt often been wrongly mistaken for objects of foreign origin), I propose to apply the name "Pseudo-nucleus." I have endeavoured to be consistent in the use of these two terms, but, as is so often the case in biological matters, there is at times a difficulty in defining a sharp boundary-line between the objects to which they are respectively applied.

A. *Muscle-Pearls.*

I have set out above (p. 267) Professor Herdman's views on the nature and origin of these. Briefly recapitulated, they are the following. From some unknown cause, minute calcareous

* Rubbel (34 a) applies the term "Mantelperlen" to these bodies, a term which I prefer to mine, though it is too late to alter the nomenclature in this paper.

depositions or calcospherules * arise in the tissues, close to the attachments of the muscles to the shell. Ectoderm-cells may "migrate to the source of irritation, and thus be responsible for the deposition of a pearl." No explanation of the origin of these calcospherules is given, but Mr. Southwell thinks it is "almost certain that they are depositions from the blood," and refers to them elsewhere as "of excretory origin" (42).

I have been led by my observations to take a quite different view of these "calcospherules" †, and as their origin is so closely related to that of Muscle-Pearls, I cannot do better than begin the present section of my paper with an account of their structure and origin.

According to my view, Prof. Herdman's "calcospherules" are not free concretions at all, but are minute pearls, composed of hypostracum; and I propose, therefore, to call them "hypostracum muscle-pearls," to separate them from "nacreous muscle-pearls." As stated by Herdman, these bodies occur close under the epidermis (unless secondarily displaced, *e. g.* by the addition of new ones), and I usually find them in the region where the muscle-attachment epithelium passes over into the ordinary shell-secreting epidermis of the mantle. A group of these hypostracum-pearls is shown on Pl. XXXIX. fig. 22, which represents a portion of the mantle-musculature of one of the unlabelled specimens in the British Museum, examined entire in oil of cloves. The same pearls, decalcified, are seen in fig. 23. These little bodies measured from 0.02 to 0.5 mm. in diameter. In Pl. XXXVIII. fig. 19 similar bodies, *hyp.*, are seen in a section along with ordinary nacreous muscle-pearls; while single individuals are shown in Pl. XXXIX. figs. 21 & 21 *a* and Pl. XL. fig. 25. Sections ground from these bodies, or cut from the organic residues left when they are decalcified, show them to be composed of the same substance as the hypostracum of the shell. They consist of calcium carbonate, in fine fibrocrystalline form, showing radial and also concentric markings, with a small central cavity (Pl. XXXIX. fig. 21 *a*). Decalcified they also resemble hypostracum in all details of structure and reaction to stains (fig. 21). Their organic basis stains more blue with hæmatoxylin than the organic parts of the other shell-substances, and takes up carmine more deeply. Their alveolar structure is also much finer than that usually found in the columnar varieties of repair-substance, so fine, in fact, that in surface-sections the reticular structure seems almost like that of the protoplasm itself. As has been observed in the hypostracum of the shell, this substance sometimes passes over into nacreous conchyolin laterally. The

* This word is presumably intended to convey the same idea as the word "concretion" adopted by me (25) in 1902, *i. e.* a sphaerocrystal-like body arising in the tissues otherwise than by epidermal secretion; and therefore analogous to cholesterol calculi, etc. (*cf.* Harting's "Calcosphærites," 12).

† I find that Rübhel (34 *a*), working on the freshwater Pearl-Mussel, *Margaritana*, has arrived independently at the same view of the nature of these bodies as that here propounded.

central cavity of a hypostracum-pearl may contain granules of doubtful origin, as in the case shown in fig. 21, but it is frequently quite empty. At times the organic basis of one of these hypostracum-pearls, when decalcified, shows a tendency to break up into segments, especially at its inner surface; the segments in such cases probably correspond to the outlines of the original secreting-cells; indeed, in such cases the whole body may have an almost cellular appearance, which is not surprising in view of the generally accepted theory that the hypostracum arises by direct transformation of the muscle-attachment epidermis.

These hypostracum-pearls shrink, on decalcification, to about one-third of their original diameters (Pl. XXXIX. figs. 22 & 23). When the tissue is old and defectively preserved, as in this preparation and in that shown in fig. 19, they come away from the wall of the enclosing sac during decalcification; but in better-preserved material, where the connection between the muscle-attachment epithelium and the pearl is maintained, the organic basis of the decalcified hypostracum-pearl remains attached to the wall of the sac (Pl. XXXV. fig. 8). Nacreous pearls, on the other hand, almost always shrink away from the sac on decalcification.

The smallest of these hypostracum-pearls that I observed measured about 0.02 mm. in diameter.

As the muscle-attachment epithelium, in *Margaritifera* at any rate, takes at most a very small part in shell-thickening, the size of these hypostracum-pearls is limited by the maximum thickness to which hypostracum normally attains. For further growth to occur, resulting in the formation of a nacreous muscle-pearl, it is necessary for some of the nacre-secreting epidermis to be present also (Pl. XXXV. figs. 8, s., & 9; Pl. XXXVI. fig. 10, sac.). Fig. 8, from a specimen given to me by Prof. Herdman, shows above a nacreous muscle-pearl and below a hypostracum muscle-pearl. Here we have a cyst, which is more or less spherical, and contains a large central cavity lined with a substance which is indistinguishable from the organic basis of hypostracum. Where an epithelium can be detected in the wall of the cyst (*m.ep.*) it possesses all the characters of muscle-attachment epithelium, its cells being continuous with the muscle-fibres, *m.*, on the one hand, and with the hypostracum, *hy.*, on the other. In some cases the muscle-attachment epithelium can be traced on all sides of the sac; in others, as in fig. 8 and fig. 10, c., only at certain parts. In still others no such epidermis is recognisable. I think, however, it is safe to assume, whether the hypostracum-pearl is surrounded by a sac of attachment-epidermis or not, that such a pearl can only arise where such a sac is present; and it is easy to detect the epithelium in most of the better-preserved examples (figs. 8, 9, & 10, *m.ep.*). Still, in some of the fairly well-preserved preparations I can identify no such epithelium. This is the case in Pl. XXXVI. fig. 11. In this example, which is on one of Prof. Herdman's slides, the hypostracum-pearl, which measures

80 μ in diameter, and has a wall about 10 μ thick, lies close to a nacreous muscle-pearl, about 1 mm. in diameter, the sac of which is shown at *ep.p.s.* The cyst is embedded in a strand of muscle traversing the mantle-parenchyma obliquely, and ending in muscle-attachment epidermis which was attached to the shell. (Such connections between the general musculature of the mantle and the shell occur here and there quite apart from the more regular muscle-scars. For examples of this in *Mytilus* see List, 27 b, Pl. 8. fig. 1.) The cyst contains at one point a little granular mass. The muscle-fibres here appear to be in direct contact with the hypostracum. The easiest explanation of this condition would seem to be the hypothesis that the original epithelium has disappeared. It is not difficult to suppose that a highly specialised "tendinous" epithelium, like the attachment-epidermis, whose fate seems to be to become a part of the shell, is incapable of regenerating itself, and, therefore, destined to die and disappear on ceasing to be functional. If we take this view, the typical hypostracum-pearl is not so much a stage in the development of a nacreous pearl as a phase parallel with it; the latter arising when the original sac contains some of the ordinary nacre-secreting epidermis, or cells capable of giving rise thereto, the former when it is composed of attachment-epithelium alone. The hypostracum-pearl would thus have a limited growth, the nacreous pearl an unlimited growth. However, in considering these cases where there does not appear to be any attachment-epithelium, it must be remembered that this particular epithelium is often very difficult to see, so that some workers have even failed to detect its existence on the regular muscle-insertions. Much light can no doubt be thrown on these questions by a really thorough study of the behaviour of the cells at the places where the muscle-attachment epithelium goes over into the ordinary epidermis of the mantle, and of the histological phenomena associated with the wandering of the muscle-attachment. The material of the pearl-oyster that I have examined so far is not sufficiently well preserved to allow of such study. So far as I know, this important matter has never been properly investigated in any mollusc.

I will now pass from the hypostracum muscle-pearls to the nacreous muscle-pearls. Typical instances of these are shown in Pl. XXXV. figs. 8 & 9 and Pl. XXXVI. fig. 10. These three examples are all explicable as derivatives of the hypostracum-pearl. Figs. 8 & 10 obviously lie in the borderland between one of the regular muscles and the parenchyma (fig. 8 is at the insertion of one of the pedal levators). Fig. 9, from one of Prof. Herdman's slides, is in a place in the free mantle where a few small muscle-strands (*musc.*) are attached to the shell. The sac of each of these pearls is lined in part by ordinary nacre-secreting epithelium, underlying which is the typical granular parenchyma, in part by muscle-attachment epithelium, continuous with the musculature. As the former is much more active than the latter, these pearls are all eccentric in shape, having a hilum

of hypostracum at one side, which, unlike the nacre, does not increase appreciably in thickness. The centre of each is a cavity, which in figs. 8 & 10 is obviously lined with hypostracum, and this hypostracum is connected by a plug of the same substance with the remaining muscle-attachment epithelium. In fig. 9 the growth of the nacre has pulled down the plug of muscle-attachment epithelium into the hilum, and produced quite a long strand of hypostracum-like substance. The presence of these hila, together with the effect of the mutual pressure of muscle-pearls when crowded together, has much to do with the generally irregular shape of commercial seed-pearls. Fig. 10, also from one of Prof. Herdman's slides, shows a very early stage in such a muscle-pearl, with a small cyst-like hypostracum-pearl alongside it. Here the ordinary epithelium of the sac seems to be gaining on the muscle-attachment epithelium.

These muscle-pearls always contain a central cavity, which may be broken up by trabeculae of hypostracum-like substance or of conchyolin, this substance being continuous with that forming the lining of the cavity. The cavity, like that of the pure hypostracum-pearl, may be empty or may contain more or less granular matter.

Muscle-pearls are often clustered and may be very numerous. Thus the old unlabelled material in the British Museum has dense clusters of these pearls in some places, and so has some of Dr. Kelaart's material.

It is by no means the case that muscle-attachment epithelium always persists in the sac of a muscle-pearl. The whole sac may pass over at an early stage into nacre-secreting epithelium, a process which is, perhaps, analogous to what occurs in the wake of an advancing muscle in the growing shell. This was apparently the case with the pearl that occupied the sac adjoining the body shown on Pl. XXXVI. fig. 11. The nucleus of this pearl is shown at fig. 12 on the same plate. The central portion of this pearl is composed of irregular conchyolin-like substance, which cannot be identified as hypostracum, and which quickly gives place to ordinary nacreous substance (*n.*). In the neighbourhood of this pearl is another, not figured here, the centre of which was comparable to the pearl shown on Pl. XXXV. fig. 8; this pearl had become more spherical secondarily by the disappearance, in the course of its growth, of the muscle-attachment epithelium. It is hard to conceive that these two pearls, and the hypostracum-pearl associated with them, are not all of similar origin.

Pl. XXXVI. fig. 13 shows the centre of another pearl, perhaps a muscle-pearl, from the mantle-margin, in one of Prof. Herdman's slides. This pearl appears to have measured about 2 mm. in diameter. The central cavity is about 0.1 mm. in its greatest diameter and is lined by abnormally thick conchyolin-like substance. Outside this are layers of ordinary nacre, which pass over into a form of repair-nacre showing radial markings,

probably due to variations in the rate of secretion of the constituent substances. This zone is 0.03 mm. thick. The central cavity is empty, except for a few granules.

In this case the muscle-pearl, if such it is, does not contain a pseudo-nucleus composed of hypostracum or a sphaerocrystal-like body such as those shown in figs. 19 & 20; and the real "nucleus" of such a pearl might be said to be a *cavity* which may or may not contain a few indistinct granules, perhaps of foreign origin.

The same condition is also typical of those pearls which I have examined from Dr. Kelaart's material. I have decalcified 38 of these in all (Pl. XXXVII. figs. 14, 15, & 16; Pl. XXXVIII. figs. 17 & 18; Pl. XLIV. figs. 46, 46 *a*, 47, 47 *a*, & 48). That these pearls are of the same nature as the other muscle-pearls seems probable from the fact that a few hypostracum-pearls occur mixed with the other pearls in Dr. Kelaart's specimens, and from the complete series of intergradations between the various forms described above, which is shown by the unlabelled specimens in the British Museum, described below. Pl. XXXVII. fig. 14 is a section of Dr. Kelaart's specimen showing "pearls in ovary." Each of these pearls lies in a cavity which doubtless was originally lined with an epidermal epithelium, though this can no longer be recognised owing to the state of preservation. The cavity is surrounded in every case by a layer of the granular subepidermal parenchyma (*par.*). Some of the pearls have been forced out of the subepidermal layer, and now lie embedded in the deeper connective-tissue, in which are seen muscle-bundles and tubules of the ovary.

In each case the centre of the pearl is a small cavity, containing a few granules or strands of what appears to be conchyolin; but the pearl in the lower right-hand corner contains also some columnar substance. The irregular conchyolin-like matter is well seen in the centre of the pearl in the top left-hand corner of the sketch, which is shown enlarged in fig. 15. It is interesting to note that the series of sections from which these drawings were made contained an example of the smaller Cestode larva, *Tylocephalum minus*.

Plate XLIV. figs. 46, 46 *a*, & 47, 47 *a* show two pearls picked from one of Dr. Kelaart's specimens, decalcified, and examined whole in oil of cloves (46 & 47) and after being sectioned (46 *a* & 47 *a*). Fig. 46 shows a dense central mass, of closely laminated nacreous substance, which on superficial examination might be taken for the remains of a dead parasite, but a section shows that the whole pearl is composed of nacreous substance around a small central cavity.

Fig. 47, examined whole, was extremely suggestive of a dead parasite; indeed, the concentric lamination of the pseudo-nucleus was not disclosed till sections were cut. These (fig. 47 *a*), however, furnished the explanation. The real centre of the pearl was, as in the rest of Dr. Kelaart's material, a nacreous

pearly mass, with a central cavity, showing at one side a plug of conchyolin-like substance. External to the normal central nacre were some irregular layers, such as one gets on the inner surface of the shell when a dark blotch or blister is caused by derangement of the secreting epithelium (compare the "Ölflecken" in *Margaritana*, Rubbel, 34 a). The opaque character of these layers, some of which were brown through the immigration or infiltration of what appeared to be cellular matter, others distinctly columnar (repair-substance), rendered the real nature of the pseudo-nucleus obscure till sections were cut. Outside these abnormal and pathological layers typical nacre was subsequently produced, thus giving a normal pearl with a dark centre.

The same characters are shown on Pl. XLIV. fig. 48, where the centre of the pearl appears opaque and granular for a similar reason. In this case the pseudo-nucleus measured about 5 mm. in diameter, and, examined entire, might have been taken for a dead parasite. It was such a nucleus, coupled with the presence of Trematodes, probably *Mutua margaritifera* Shipley & Hornell, in the tissues of Dr. Kelaart's pearl-oysters, that, in 1901, led me to the probably mistaken conclusion that a Trematode might be one of the organisms which afford the stimulus for the formation of the pearl-sac in *Margaritifera vulgaris*, as the Trematode *Gymnophallus* does in *Mytilus* * (25, p. 162).

But examination of sections (Pl. XXXVII. fig. 16 and Pl. XXXVIII. fig. 17) showed that the opaque pseudo-nucleus was due to a break in the continuity of the nacre; a layer of granular substance (*gr.*), apparently dead cells (perhaps of the nature of the "oil-spots" in *Margaritana*, or derived from an immigration of leucocytes such as Moynier de Villepoix observed (28, p. 112) or from Protozoan parasites), being followed by the secretion of two horny layers of amorphous repair-substance (*am.*, *am.*'), after which normal nacre resumed its development. The real centre of this pearl, as in the rest of Kelaart's material, consists (Pl. XXXVIII. fig. 18) of shreds of conchyolin-like material, and a few obscure granules, in a cavity which is surrounded first by granular repair-nacre and then by ordinary nacre.

In the two unlabelled specimens in the British Museum, from which Pls. XXXVIII.-XL. figs. 19-28 and Pl. XLIV. fig. 49 are drawn, while the pearls agree with those described above in their clustered habit, occurrence in the muscular regions, and association with hypostracum-pearls, we have more frequently as nuclei either hypostracum or special sphærocrystal-like bodies, which I regard as formed of columnar repair-substance.

* Fuller knowledge and closer study lead me to doubt the accuracy of my own observations as to the occurrence of the remains of Trematodes in the pearls produced by the other species of molluscs referred to on p. 162 of my 1902 paper with the exception, of course, of *Mytilus edulis*, in which the relation between Trematodes and pearls is beyond question.

Pl. XXXVIII. fig. 19 shows a group of such pearls, scattered among which are hypostracum-pearls (*hy.p.*). The nucleus of the eccentric pearl at *nu.* is clearly composed, like that of the incipient pearl shown on Pl. XXXIX. fig. 22, *nuc.*, of hypostracum, and resembles the hypostracum-pearl shown at fig. 21, which has a small quantity of granular contents. One of the columnar nuclei is shown enlarged at fig. 20. This is the nucleus of the large pearl shown in the upper part of fig. 19 (Pl. XXXVIII.). Centrally there is a small cavity, in which a granular mass of doubtful origin is noticeable (*gr.*). This cavity is surrounded by an irregular zone of substance which seems to be the same as the amorphous repair-substance of the shell (*cf.* Pl. XL. fig. 29). Externally to this there is columnar substance, forming a sort of sphaerocrystal-like mass, the fibrocrystalline calcium carbonate being deposited in a conchyolin-like basis, which, on decalcification of the pearl, remains as a framework. This substance is seen cut tangentially at one end of the pseudo-nucleus. Outside this is the normal nacre of the pearl. In one or two cases the amount of granular matter in the centre of the pearl was considerable; thus Pl. XL. fig. 24, which is taken from the same series as the preceding figures, shows the central portion of a pearl which has a dumbbell-shaped double nucleus, the larger half being about .17 mm. in diameter, and each half containing an opaque brownish mass of dead animal matter, perhaps of cellular origin, but quite unrecognisable. The contents of the two cavities were continuous at one point. There is nothing to suggest that either of these bodies represented a dead Cestode; I could find no denser portion, such as would naturally occur at the point representing the myzorhynchus, nor was there anything that could be safely identified as the cuticle of the parasite. If the contents suggested anything, it was rather the remains of a large Protozoan parasite, containing spore-like bodies in a plasma (like the Sporozoan spores which Dubois claims to have identified in the nucleus of a pearl from *M. vulgaris* from the Mediterranean, 7, p. 311, and 8, p. 104); or, perhaps, a mass of mucus containing a few cells. Each of these centres was surrounded immediately by the amorphous substance, this being followed by, and continuous with, a radially calcified layer, the bases of the calcareous prisms being evidently embedded in the amorphous substance, which extended up between them to form the organic framework of the columnar substance. This condition is comparable to that seen at *tr.* on Pl. XLI. fig. 30, which shows the amorphous substance secreted at the junction of two pearls in a compound pearl, passing over into columnar repair-substance.

Externally to the columnar layer of the pseudo-nucleus the normal nacreous layers of the pearl are formed.

The other extreme is shown on Pl. XL. fig. 25, a hypostracum-pearl .08 mm. in diameter, the central cavity of which has no contents whatever.